March 1961
VOLUME 83/NUMBER 3

THE AMERICAN REVIEW OF Respiratory Diseases

OFFICIAL JOURNAL OF THE AMERICAN THORACIC SOCIETY
Medical Section of the National Tuberculosis Association

1961 ANNUAL MEETING

CINCINNATI, OHIO

MAY 21–25

The 1961 Annual Meeting of the National Tuberculosis Association, the American Thoracic Society, and the National Conference of Tuberculosis Workers will be held in Cincinnati, Ohio, May 21–25.

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Exhibits will open at 8:30 a.m., Monday, May 22, and will be on view, during morning and afternoon hours, throughout the meeting. The high caliber of exhibits, as at previous meetings, will make this a phase of the program to which all attending will want to devote a generous portion of their time.

Preceding the general meetings—ATS Committee and Council meetings will be held Saturday and Sunday, May 20 and 21. The annual Tuberculosis Nursing Advisory Service Seminar will be held on Saturday, May 20. There will be special interest sessions on Sunday, May 21.

Medical, public health, and nursing sessions will begin on Monday, May 22. General sessions will include an opening program Monday at 9:00 a.m. A keynote, awards, and NTA business session will be held Monday evening, and a closing general session at 11:00 a.m., Thursday, May 25.

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MARCH 1961
VOLUME 83/NUMBER 3

THE AMERICAN REVIEW OF Respiratory Diseases

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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2		0		1		1		0		0		2		100–200,000	
3		6		0		0		0		1		4		200–500,000	
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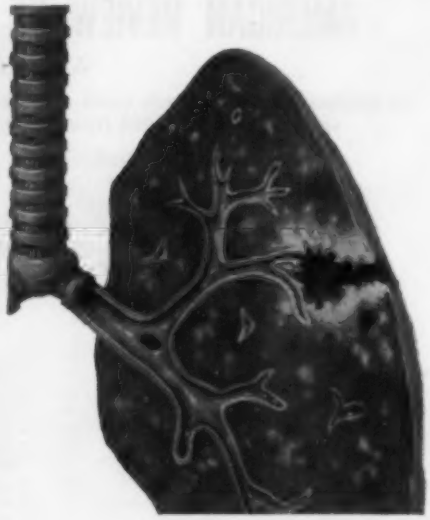

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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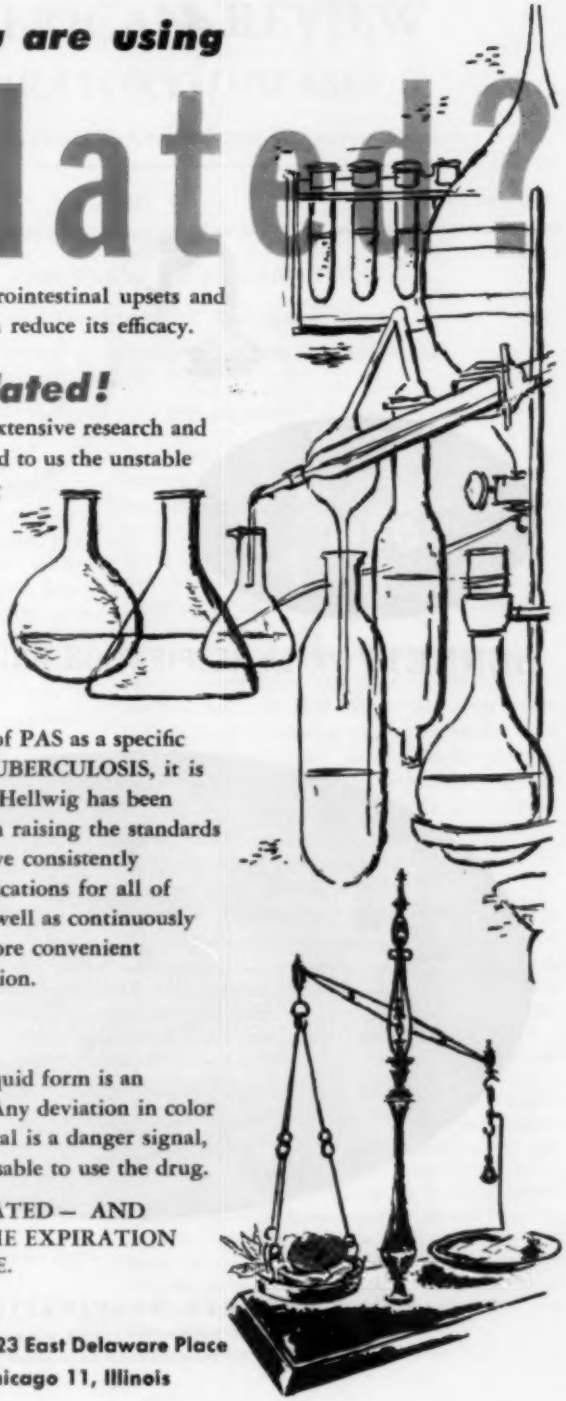

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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 83 *March 1961* NUMBER 3

THE CHANGING SPECTRUM OF NOCARDIOSIS[1, 2, 3]

A Review and Presentation of Nine Cases

JOHN F. MURRAY, SYDNEY M. FINEGOLD, SEYMOUR FROMAN, AND DRAKE W. WILL

(Received for publication July 28, 1960)

Introduction

Nocardiosis is a localized or disseminated infection which may involve all tissues and is produced by members of the genus Nocardia, principally *Nocardia asteroides*. Other species, notably *Nocardia brasiliensis*, have been implicated, but *N. asteroides* appears to be the overwhelmingly predominant human pathogen identified in the United States and in Europe. Delays in the early diagnosis and treatment of nocardiosis are often due to confusion with pulmonary tuberculosis, the systemic mycoses, and the closely related actinomycosis. In the laboratory, cultures may be discarded too early or mistaken for saprophytic acid-fast bacilli. Correct diagnosis is imperative because nocardiosis is susceptible to sulfonamide therapy but refractory to chemotherapeutic regimens now in use for tuberculous, most bacterial, and fungous infections.

Once considered to be extremely rare, both the localized and disseminated forms of nocardiosis appear to be increasing in incidence; this may be due in part to greater facility in laboratory recognition of the causative organism. However, numbers of multiple case reports (1–3) in the recent literature would suggest that the over-all incidence of the disease is actually rising. In contradistinction to tuberculosis and other fungous infections, little attention has been given to nocardiosis as one of the penalities of sustained steroid therapy as well as its recognition as an infection with a probable association with neoplastic disorders.

This report will review some fundamental aspects of nocardiosis, with emphasis on diagnostic methods and treatment. The writers' experiences with 9 cases of nocardiosis are presented to illustrate the vagaries of the disease and to demonstrate its association with underlying disorders of the immune mechanism and adrenocortical-steroid therapy.

Review of Literature

The first description of disease produced by aerobic actinomycetes was written in 1888 by Nocard (4) who had studied farcy (glanders) in cattle on the island of Guadeloupe. Eppinger's description (5) of so-called pseudotuberculosis produced by similar aerobic branching organisms in man is considered to be the first report of human nocardiosis. Between the publication and the time of preparation of the present report (1960), 179 cases of nocardiosis have been reported in the literature with sufficient bacteriologic information to allow recognition of the causative organisms. Many writers have failed to distinguish between actinomycosis and nocardiosis clinically and/or bacteriologically. Although no documented cases have been found in the Oriental literature, nocardiosis has been recognized throughout the rest of the world; however, most reports have originated from the United States and Europe.

Sex and age incidence: In 155 cases in which the

[1] From the departments of Medicine, University of California Medical Center and the Veterans Administration Center, Los Angeles, California; Olive View Hospital, California; and the Department of Pathology, University of California Medical Center, Los Angeles, California.

[2] Supported in part by U. S. Public Health Service Grant H-1004.

[3] Presented in part before the Medical Session, as part of *Section 4C*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 17, 1960.

sex of patients is given, 70 per cent have been males. All age groups may be involved with reported cases ranging from a four-month-old child (6) to an eighty-seven-year-old man (7). The majority of cases are found in the twenty- to sixty-year age group.

Mortality: The over-all mortality in all reported cases is 54 per cent. Sulfonamide therapy was first used in 1937, although unsuccessfully, by Alestra and Girolami (8). Effective treatment with sulfonamides was not reported until 1943 by Lyons and his coworkers (9) who used sulfadiazine as the only chemotherapeutic agent in a woman with severe subcutaneous nocardiosis. In the pre-sulfonamide period the mortality was approximately 75 per cent; since the advent of sulfonamide therapy the mortality has fallen to 46 per cent. Even the latter figure is misleading, since an increasing number of cases are being reported in which nocardiosis is incident to neoplastic disorders, with death primarily caused by the underlying disease. At times, however, the inability to make an early diagnosis and institute appropriate treatment has apparently contributed to the patient's death.

Site of infection: The great majority of cases of nocardiosis are pulmonary; 31 per cent of these remain confined to the respiratory tract with no evidence of dissemination or multiple system involvement. A small percentage (5 per cent) present with central nervous system involvement alone, and a slightly higher number of cases are described in which only the pulmonary and central nervous systems are involved. Nocardiosis of skin and subcutaneous tissues alone, excluding Madura foot, occurs in 14.5 per cent of the total cases. It is not commonly recognized that generalized or disseminated nocardiosis is almost as frequent as pure pulmonary disease, occurring in 30 per cent of all patients. The following structures may be affected (in order of frequency): lung, skin and subcutaneous tissue, brain, pleura, spleen, kidney, heart, liver, bones and joints, peritoneum, lymph nodes, adrenals, gastrointestinal tract, meninges, pancreas, thyroid, eye, ear, marrow, endocardium, spinal cord, pituitary, and bladder. The largest number of cases of disseminated disease also show pulmonary infection. Occasionally organisms are isolated from blood cultures.

Association with other diseases: Nocardiosis is occasionally associated with other diseases, and this association is slowly increasing, particularly in those chronic diseases in which long-term steroid therapy is used. These include: leukemia (2), Hodgkin's disease (2, 10), lupus erythematosus (11), asthma (12), pemphigus vulgaris (13), and pulmonary alveolar proteinosis (14). Carcinoma of the breast (2), pulmonary tuberculosis (15), emphysema and bronchiectasis (16), anthracosilicosis (17), war wounds (18), pilonidal sinus (16), Paget's disease of bone (19), and dysproteinemia (20) have also been reported as diseases associated with nocardiosis.

Bacteriologic and Laboratory Aspects

The genus Nocardia (also called Proactinomyces in much of the current literature) is a member of the large group of organisms included in the order Actinomycetales. These are transitional forms which show certain features distinguishing them from true bacteria and true fungi.

The pathogenic nocardiae are aerobic, grow slowly but moderately well on most laboratory media, and produce pigmented colonies. Although few of the organisms withstand the routine sputum concentration procedures used in the cultivation of *Mycobacterium tuberculosis* (21), except for the sodium phosphate techniques, most grow satisfactorily on the egg-glycerol media with malachite green used for tuberculosis culture. Distinctive filaments, exhibiting true branching, are present in early cultures, and later these tend to fragment, producing bacillary and coccoidal forms. Acid-fastness, particularly in *N. asteroides*, is variable, being most prominent in tissue and less evident in culture. Gordon and Mihm (22), in a study of 98 strains of *N. asteroides*, found marked variation in acid-fastness, and McClung and Uesaka (23) have called attention to the influence of many environmental factors on the acid-fastness of the Nocardia. Rarely, species of Nocardia show motility (24). The organisms are gram-positive, producing considerable numbers of beaded forms. The filaments are fragile, and routine smears prepared from growing cultures may be difficult to interpret since the demonstration of branching is of paramount importance. Huppert and co-workers (25) have proposed slide culture as a useful diagnostic method for the early determination of branching. Using corn meal agar in Littman culture slides, they showed that, although some mycobacteria produced short single-branched forms, Nocardia alone showed long filaments with several branches. The technique is both simple and reliable and should be used in the diagnostic study of such organisms.

On most media, colonies of the pathogenic Nocardia vary widely in appearance, consistency, and pigmentation. Since colonial forms resemble many of the saprophytic mycobacteria both in appearance and pigmentation, the observation of branching filaments is necessary for differentiation; true branching is not seen in the mycobacteria. In fluid media, growth occurs as a pellicle on the surface of the medium or as a sediment, and Bojalil and Cerbón (26) differentiate some pathogenic nocardiae by their growth in fluid media.

For isolation and diagnostic purposes, blood and Sabouraud agar medium provide excellent substrates for growth of the pathogenic Nocardia, and these organisms grow well at 37°C. Stock cultures may require temperatures of 35° or 28°C. (22). Growth from clinical materials is poorer on the inhibitory media used for the primary isolation of pathogenic fungi. Media containing chloramphenicol (Mycosel agar), penicillin, and streptomycin or other antimicrobials should not be used.

The speciation of the Nocardia is a difficult task. Gordon and Mihm (22), Schneidau and Shaffer (27), Bojalil (26), and others have made admirable attempts to accomplish speciation by cultural and physiologic means, but even these detailed studies show some variance in results. Part of the difficulty is because continued laboratory cultivation or storage of any of the genera of Actinomycetales increases the tendency to show variation. The taxonomists, therefore, in their search for stable characteristics among the Nocardia, favor a wide variety of culture media for colonial observation and propose a battery of physiologic determinations for speciation in this exceptionally difficult group of organisms.

Serology and Immunology

The difficulties encountered in preparing satisfactory antigens for serologic tests from the mycelial growth of actinomycetes has prohibited extensive serologic studies with these organisms. Ludwig and Hutchinson (28), using sonically disrupted suspensions, were able to produce antisera in rabbits for agglutination and precipitin testing which showed a moderately high degree of specificity. Nocardial antigens prepared by them showed some cross reactions, however, only in low titers. Recently, Affronti (29) prepared purified protein derivatives (PPD) from *N. asteroides* and several unclassified acid-fast bacilli; he demonstrated cross reactions in low dilutions by precipitin tests between all the preparations used, but found specificity at high titers. Nocardia PPD and PPD-S preparations consistently produced small fading skin reactions in animals previously sensitized with the homologous Nocardia strain employed (*N. asteroides*).

No attempts to demonstrate neutralizing, agglutinating, precipitating, or complement-fixing antibodies in human nocardiosis have been reported. Drake and Henrici (30), however, in a now classical study, conducted extensive experiments which showed that *N. asteroides*, the principal species isolated from human infections, would produce delayed dermal sensitivity in animals and that the reactions to their antigens were specific with no demonstrable cross reactions with tuberculins.

Drug Susceptibility

Reliance upon *in vitro* susceptibility to chemotherapeutic agents has led in the past to errors in the choice of agents for the therapy of nocardiosis. Strauss, Kligman, and Pillsbury (31) found that chlortetracycline, oxytetracycline, chloramphenicol, and sulfadiazine inhibited the growth of Nocardia in culture, with sulfadiazine the least effective of the four agents. In experimental infections in mice, however, sulfadiazine conferred 100 per cent protection against the development of the disease, whereas chlortetracycline and chloramphenicol were only partially effective. Runyon (32) found similar results and, in addition, demonstrated that penicillin was inactive *in vitro* and *in vivo*. *In vitro* studies with sulfonamides are especially unreliable. Recently, Larsen (2) and her co-workers, studying freshly isolated strains from human disease, found marked variation in susceptibility to a wide range of antimicrobials in *in vitro* testing.

The results of both experimental and clinical studies lead to the conclusion that *in vitro* drug-susceptibility tests are not accurate guides to the therapy of nocardiosis. Sulfonamides are the only drugs that have proved effective in several *in vivo* experiments. The results with antimicrobials are variable and conflicting.

Treatment

It follows from the above statements that by far the most important agents for nocardiosis are sulfonamides, irrespective of drug-susceptibility tests. The ineffectiveness of sulfonamides and the successful use of penicillin and other antimicrobials may represent failure to distinguish between nocardiosis and actinomycosis. Sulfadiazine has been successfully used on many occasions; dosages to maintain a serum concentration of 8–12 mg. per 100 ml. are recommended. Although there has been considerably less experience with other sulfonamide preparations, any of the available drugs giving adequate, sustained blood concentrations should work equally well. It is uncertain whether any additional benefit is attained by combining other chemotherapy with sulfonamides. Selection of which of the available antimicrobials to use in combination is difficult, and review of the literature does not reconcile the problem.

Besides success with sulfonamides (alone or combined), there are cases reported of proved nocardiosis in which the patients have responded to chloramphenicol (33), penicillin and chlortetracycline (34), and penicillin and streptomycin (35).

It is possible that some of these responses may have been spontaneous. Because careful *in vivo* studies have repeatedly demonstrated a failure of penicillin to protect against experimental infections, it is difficult to accept the recent recommendation that penicillin should be selected for adjuvant therapy (36). If combined therapy is preferred, it seems reasonable to use chloramphenicol or one of the tetracycline drugs. The duration of necessary chemotherapy varies with the type of nocardiosis under treatment. Sulfonamides should be given for at least several months in all cases of nocardiosis, and some patients with indolent, progressive disease may require a year or more of therapy.

An indispensable aspect of the treatment of nocardiosis is adequate surgical drainage of purulent collections and excision of all necrotic tissue. There are many reports of survival following combined surgical and drug therapy of extensive abscesses in the brain, subcutaneous tissues, and chest.

Pathology

Pathologically, *N. asteroides* infections are characterized by acute necrosis with abscess formation in all tissues. In the lungs, many small nodules may be formed which show central necrosis and peripheral acute inflammatory exudate with fibrin and little or no evidence of fibrosis. Organizing and organized exudate usually surrounds the nodules; these may become confluent, with the occasional formation of large cavities. Similar abscesses may be found in the brain and other viscera. Tuberculoid granulomas and Langhans' giant cells have been described but appear to occur infrequently. Fibrosis is seldom seen in generalized nocardiosis except in subcutaneous and pleural disease, in which it may be extensive. Little is known of the pathology of the resolved, successfully treated disease.

Organisms may be seen in tissues stained with Gram or acid-fast stains; they usually appear as beaded filaments which may show branching. It is impossible to distinguish Nocardia from other acid-fast organisms when only bacillary forms are present; branching is necessary to identify the organism. Nonacid-fast branching forms or sulfur granules may be found in both actinomycosis and nocardiosis. Nocardia are not seen in hematoxylin-eosin-stained preparations or in sections prepared with Schiff reagent stains to demonstrate fungi.

Animal Pathogenicity

Henrici and Drake (30) found *N. asteroides* to have little invasive power for rabbits and guinea pigs. Large doses of organisms produced death acutely, particularly if given intravenously; but small doses produced no chronic progressive disease. Strauss (31) and Runyon (32) were able to produce progressive disease in mice, and this animal has been used for most of the *in vivo* susceptibility studies thus far reported.

Case Reports

Case 1. J.W., a thirty-six-year-old, white, married cook, entered Wadsworth Veterans Hospital on April 21, 1952, complaining of left anterior chest pain, night sweats, and fever of two weeks' duration. He also developed a cough productive of small amounts of mucoid sputum which was often blood streaked. When first examined he was febrile and the chest was asymmetrical with a tender prominence of the left pectoral area. A plural friction rub was heard over the left lateral chest, and persistent moist rales were heard throughout the left midlung field. Physical examination was otherwise unremarkable. Laboratory study revealed a hemoglobin of 11.2 gm. per 100 ml. and a leukocyte count of 14,300 per cu. mm. with a shift to the left. Urinalysis was normal. A culture of pus obtained by aspiration of the left chest abscess yielded *N. asteroides*. Cultures for other organisms were negative. Roentgenographic examination revealed an infiltration involving the midportion of the left lung field (figure 1).

The patient was placed on sulfadiazine, penicillin, and chloramphenicol. Two weeks after admission, under local anesthesia, the left chest lesion was explored and a small amount of purulent material was found under the pectoralis muscle. Three weeks later because of persistent signs of infection and erosion of the second and third left anterior ribs, the patient was explored under general anesthesia. A wide exposure of the subpectoral space revealed a large amount of thick pus and a sinus tract that extended beneath the ribs into the pleural cavity. The diseased ribs were resected and, following the procedure, the patient gradually improved. He was kept on sulfadiazine alone for an additional two months and when last seen was afebrile and well.

Comment: This patient developed nocardiosis with a pneumonic infection that extended to his chest wall and subcutaneous tissue. Limited drainage failed to control his infection and extensive surgical excision of diseased tissue was required. After treatment with sulfadiazine for three months he apparently made a complete recovery.

Case 2. R.S., a thirty-seven-year-old Puerto Rican alcoholic, was admitted in a comatose state to Wadsworth Veterans Hospital on June 17, 1954. He had had convulsive episodes at approximately monthly intervals for three years and a history of having had fluid removed from the left chest three years previously. A few days prior to admission, he began to complain of headache and stiff neck. He was unable to walk the following day, rapidly

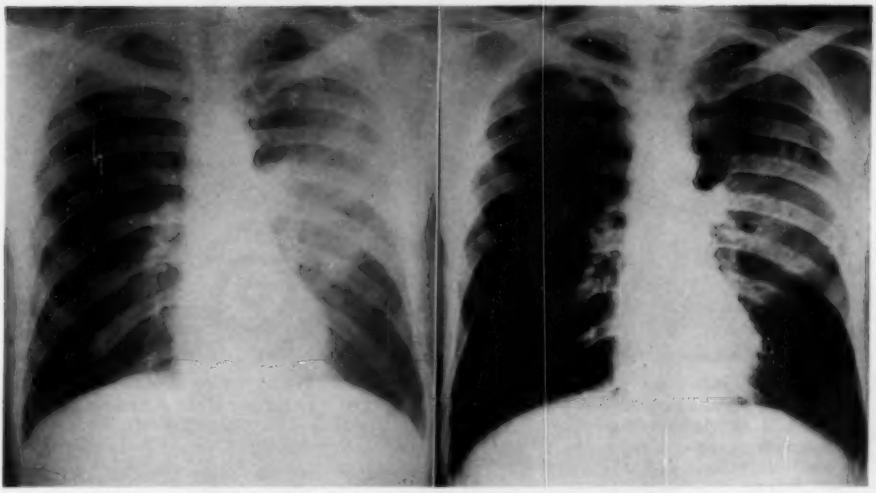

FIG. 1. Roentgenograms of *Case 1* showing: (Left) (April 25, 1952) diffuse parenchymal infiltration with probable contiguous pleural involvement; (Right) (May 28, 1952) there has been progression with formation of a small abscess cavity in the second left anterior interspace and erosion of the left second and third anterior ribs.

became stuporous, and he had numerous seizures. Physical examination revealed a slightly obese, comatose male, whose temperature was 104°F. There was marked nuchal rigidity, with the head turned to the right and a positive Babinski sign on the left. There were numerous rales at the left base, and a chest roentgenogram revealed increased markings in the left midlung field. Skull roengenograms were normal. The leukocyte count on admission was 15,200 per cu. mm. with 87 per cent polymorphonuclear leukocytes. Cerebrospinal fluid revealed 5,000 leukocytes, all of which were neutrophils, and a protein of 248 mg. per 100 ml. Cerebrospinal fluid cultures, including some for fungi and *M. tuberculosis*, were consistently negative.

The patient was initially placed on penicillin and tetracycline therapy, and two days later sulfadiazine and streptomycin were added. Within a week he was responding to questions and said he had no headache or pain. All antimicrobials were stopped two weeks after admission; however, when a lumbar puncture again revealed 1,800 leukocytes, it was decided to reinstitute the chemotherapeutic regimen. A week later, despite this therapy, the patient became increasingly febrile, more confused, and again developed neurologic abnormalities. On July 27, because of a progressive and rapidly deteriorating neurologic course, a tracheotomy was performed and burr holes were placed in both frontal and temporal regions. Cloudy ventricular fluid was obtained, but no abscess cavity was entered. The patient remained febrile and quietly died on the following day.

Autopsy revealed that the left pleural cavity was completely obliterated by adhesions and an organizing bronchopneumonia throughout both lungs. Study of the brain revealed numerous abscesses in the cerebellum, left temporal and occipital lobes, and right frontal lobe. These contained creamy, green pus from which *N. asteroides* was cultured.

Comment: This man entered the hospital with signs of acute central nervous system infection of unknown etiology. He made an initial response to multiple antimicrobials and sulfadiazine, which unfortunately were stopped too early. A relapse occurred and numerous nocardial brain abscesses were found at autopsy. It is likely that the primary infection was in his left lung although no organisms were cultured from this site.

Case 3. J.F., a forty-eight-year-old, white, male, kitchen helper, was admitted to the Los Angeles County General Hospital in August, 1954, following a progressive history of weight loss, productive cough, and weakness. A chest roentgenogram was said to be consistent with far advanced tuberculosis, and several gastric and sputum cultures and guinea pig inoculations were positive for *M. tuberculosis*. He was started on streptomycin, isoniazid, and PAS and, after the induction of a pneumoperitoneum, was transferred to Olive View Hospital on November 8, 1954, where this drug treatment was continued. In January, 1955, a mass was noted over the mid-sternum from which thick,

FIG. 2. (Left). Gross lung specimen of pulmonary nocardiosis from *Case 3*, showing bronchopneumonia, abscess formation, fibrosis, and thickened visceral and parietal pleura.

FIG. 3. (Right). Photograph of a gross brain section from *Case 3* showing soft, semifluid abscesses in the cerebral cortex. The abscesses are characteristically circumscribed and pale greenish-yellow.

purulent material was aspirated, and acid-fast organisms were recovered which were subsequently identified as *N. asteroides*.

The patient had made no apparent clinical response to his antituberculous therapy at this time. He was then treated with numerous antimicrobials and a single course of sulfadiazine for seven days, and, as shown roentgenographically, his extensive soft, linear and nodular lesions gradually improved. However, in June, 1955, his condition again deteriorated, and a new abscess and sinus tract developed over the sternum and another on the left breast. *N. asteroides* was also recovered from these sinuses. In November, 1955, the patient developed Jacksonian seizures and weakness of the right arm and leg. Cerebrospinal puncture was unrevealing. In addition to several antimicrobials, sulfathalidine therapy was instituted with apparent improvement over the following month. However, the patient again developed signs of marked central nervous system impairment and over the next few weeks had several attacks suggesting diffuse cerebral involvement. He died in January, 1956.

Autopsy revealed a small abscess of the left upper lobe communicating with a bronchopleural cutaneous fistula. There were multiple fistulous openings from empyemas on both sides of the sternum with involvement of the manubrium and right fourth rib. There was complete bilateral obliterative pleuritis except for the empyema spaces on both sides. Patchy bronchopneumonia was present in both lower lobes (figure 2). In addition, there was complete obliterative pericarditis and a mycotic infarct of the spleen. Multiple abscess cavities were found in the brain (figure 3). Cultures of the empyema and pulmonary and brain abscesses grew *N. asteroides* (figure 4). Microscopically, a bronchopneumonia with multiple abscess cavities was seen in the lungs, in which there was no evidence of caseous granulomatous disease. Numerous acute necrotic abscesses were present in the cerebral and cerebellar cortex and spleen. Gram-Weigert stains of all abscesses showed gram-positive, beaded filamentous organisms, many of which were branching. No acid-fast forms were demonstrated in any of the tissue sections.

Comment: This man had well-documented pulmonary tuberculosis which apparently coexisted with nocardiosis. His tuberculosis was apparently controlled with isoniazid, streptomycin, and PAS, but the nocardial infection gradually spread and eventually was fatal. This man should have had extensive surgical drainage and more vigorous sulfonamide therapy early in the course of his illness.

Case 4. A.A., a sixty-three-year-old chef, entered University of California at Los Angeles Medical Center for the first time on August 24, 1957. The patient was in excellent health until July, 1957, when he developed a vesicular skin eruption diagnosed as pemphigus vulgaris by biopsy. He was placed on prednisone in increasing amounts up to 160 mg. daily and later required insulin therapy. Ten days before admission he had noted swelling of the right leg and a left foot drop. Two days prior to admission he became severely depressed and confused. He had lost fifty pounds during his illness. Examination revealed a slightly obese, white male with a marked personality disorder characterized by a depressive reaction. Vital signs were normal. The skin revealed numerous erythematous papular lesions over the head, thorax, and abdomen; buccal mucosa; and nares. Some of the lesions were ulcerated. Examination of the chest revealed evidence of mild obstructive emphysema. Examination of the heart and abdomen was normal. Also

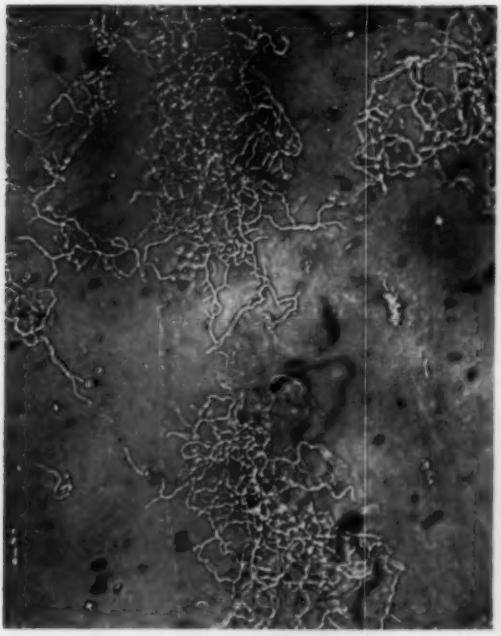

FIG. 4. Photomicrograph of a slide culture of *Nocardia asteroides* recovered from *Case 3* showing numerous branching, filamentous organisms. (Corn meal agar, 450 ×)

noted were thrombophlebitis of the right lower extremity and a left peroneal nerve palsy. The blood count on admission was normal and a urinalysis showed 4-plus sugar and a trace of acetone. Blood sugar was 480 mg. per 100 ml. The initial chest roentgenogram was normal except for slight elevation of both diaphragms. The electrocardiogram showed nonspecific ST-T wave changes.

Treatment consisted of supportive care, a gradual tapering of the steroid dosage, and insulin as needed. Four days following admission the patient developed a cough with sputum, and an infiltration was seen roentgenographically in the right chest. This increased markedly in size and within a few days involved most of the right lung with numerous abscess cavities and a pleural effusion (figure 5). The abscesses enlarged over the next few weeks and the patient had a cough productive of large amounts of grayish-yellow sputum. However, his temperature throughout this period was virtually normal, and no pathogenic organisms had appeared on cultures of sputum. He was given erythromycin, chloramphenicol, and novobiocin at various times. He subsequently developed a paralytic ileus and gastrointestinal hemorrhage, and after forty-six days of hospitalization went into circulatory collapse and died.

At autopsy he was found to have confluent acute bronchopneumonia with abscess formation and early gangrene of the right lung, bronchopneumonia of the left lung, empyema of the right pleural cavity, and abscesses of the brain, liver, and kidney. From all of these sources, a nocardial organism was cultured. Gram stain of the tissues showed gram-positive, branching, delicate, filamentous organisms consistent with nocardiae, that were nonacid-fast (figure 6). In addition, numerous sputum cultures obtained prior to death but reported afterwards contained the same organism.

Comment: This man required huge doses of prednisone to control his pemphigus vulgaris. He developed several complications of his steroid therapy, including nocardial bronchopneumonia. Features of his infection were the rapid course, extensive abscess formation, and metastatic spread to distant organs. Steroid therapy possibly contributed to the latter process as there was a noteworthy lack of an encapsulating reaction surrounding the inflammatory sites.

Case 5. A.P., a sixty-eight-year-old, white, retired brick mason, was first seen at the UCLA Medical Center on February 24, 1958. The patient was perfectly well until four years prior to his entry when he developed the onset of mild congestive heart failure which responded to treatment with digitalis, salt restriction, and occasional mer-

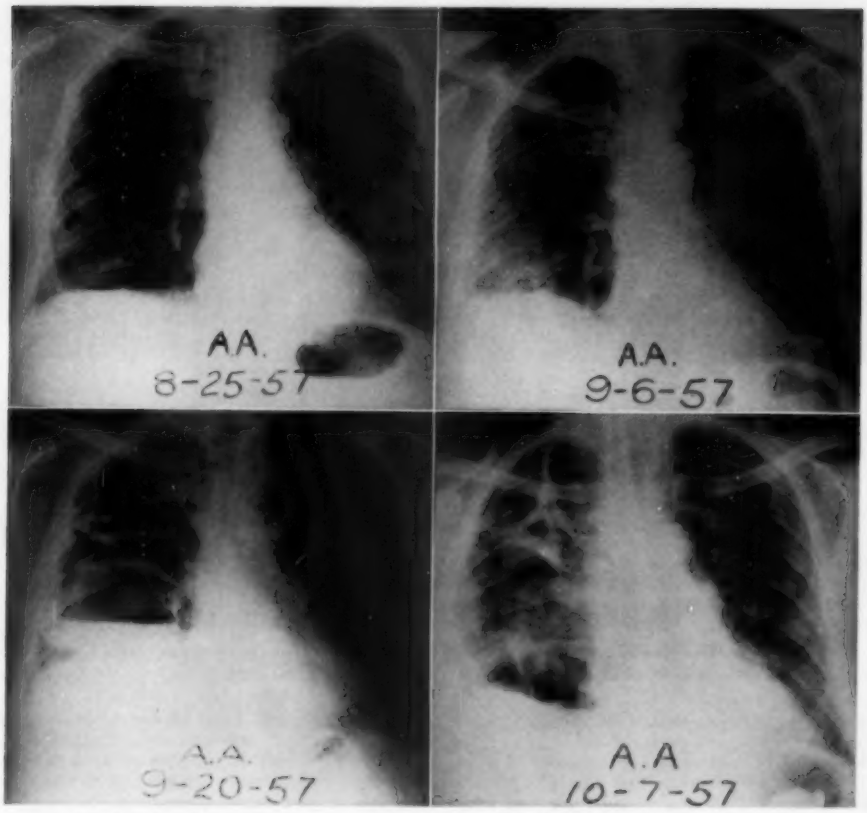

FIG. 5. A series of four roentgenograms of *Case 4* showing the swift progression of pulmonary nocardiosis. The minimal infiltration present on August 25, 1957, rapidly increased in size (September 6, 1957) with progressive and extensive destruction and abscess formation on September 20, 1957, and October 7, 1957.

curial diuretics. Approximately four months before admission, the patient had had a routine photofluorogram which was abnormal and he was referred for further evaluation. There had been no weight loss, weakness, or fatigue. Except for an occasional dry cough the patient's physical examination was entirely within normal limits. Roentgenograms from November, 1957, through April, 1958, showed a 5- to 6-cm. mass in the posterior segment of the right upper lobe that had not changed in size but, on the latest film, had a crescentic radiolucency (figure 7). Electrocardiograms showed nonspecific T-wave changes. The blood count and urinalysis were not remarkable. The patient was bronchoscoped, and no endobronchial disease was noted. Washings taken from the right bronchial tree for pathologic and cultural studies were negative. Skin tests for fungous diseases and tuberculosis were negative. Four sputum cultures were negative for acid-fast bacilli.

A preoperative diagnosis of carcinoma of the lung was made, and the patient had a right upper lobe lobectomy. Pathologic examination of the resected specimen revealed a 5- by 6-cm. mass containing a cystic cavity approximately 3 cm. in diameter. Microscopic examination of the cavity wall and adjacent lung showed a lining layer of granulation tissue with foci of epithelioid cells. There were numerous rounded granulomatous areas without caseation and giant cells. Within the abscess cavity and neighboring bronchi were clumps of finely fibrillar cotton ball-like material which consisted of dense aggregations of branching, filamentous, beaded, thin gram-positive rods. Culture of this organism showed it to be a Nocardia species which is being studied further. The patient was placed on sulfadiazine and penicillin; however, following an urticarial reaction the sulfadiazine was discontinued and streptomycin was substituted. These antimicrobials were given for a total of four

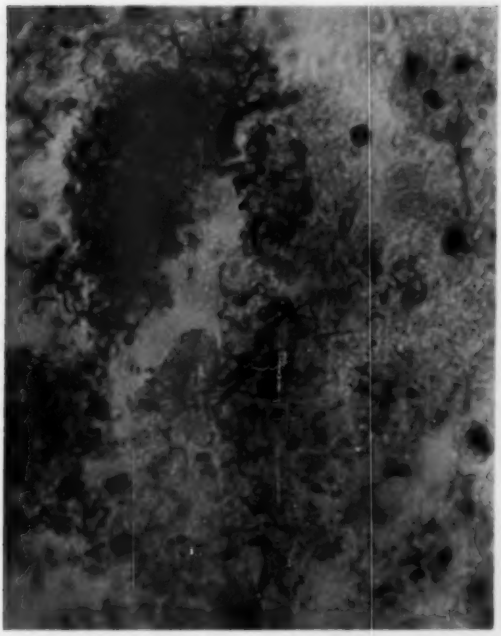

FIG. 6. Photomicrograph of a small pulmonary abscess in *Case 4* showing branching, filamentous Nocardia. (Gram stain, 450 ×)

weeks. The patient has been followed for two years postoperatively and remains clinically well except for signs of persistent congestive heart failure.

Comment: This man had a chronic lung abscess with a mycetoma due to a nocardial organism. This lesion was completely resected and there has been no evidence of recurrence in the subsequent two years. It is doubtful if the antimicrobials used contributed to his improvement and he must be considered primarily a surgical cure.

Case 6. G.S., an eighty-one-year-old, retired contractor, entered UCLA Hospital for the first time on June 24, 1958. The patient was in good health until five and one-half years prior to admission when he developed a pulmonary infection. A single sputum culture was said to show *M. tuberculosis* and he was placed on streptomycin for one year, with subjective improvement. Nine months before admission, he developed a productive cough and was given a two-month course of penicillin and streptomycin which relieved these symptoms. Three months prior to admission he had another episode of cough and sputum production which responded to penicillin therapy. He was placed on digitalis for symptoms of mild congestive heart failure. Shortly before admission, his cough had been productive of thick dark sputum, and he complained of mild exertional dyspnea.

On examination, the patient was a well-preserved elderly man in no distress. Examination of the thorax and lungs showed signs of emphysema with scattered rhonchi and inspiratory rales at the bases. There was a prominent systolic impulse in the epigastrium. No abnormal heart sounds were heard. The liver was barely palpable. The remainder of the physical examination was negative. The hemogram was normal, but a urinalysis showed leukocytes and erythrocytes and a trace of protein. An electrocardiogram was suggestive of an old posterior myocardial infarction. A chest roentgenogram showed generalized pulmonary fibrosis with thickening of the pleura, retraction of both hila, and infiltration at the right base. Two sputum cultures revealed *N. asteroides*. The patient was placed on a four-week course of tetracycline and sulfisoxazole, and the organism was not recovered from a sputum culture obtained three months later. In January, 1959, another sputum sample revealed *N. asteroides* which again responded to the same antimicrobials for one month. However, repeat sputum studies during exacerbations of cough and expectoration in August, 1959, and January, 1960, again grew *N. asteroides*. The patient is currently being given his third course of sulfadiazine which this time will be given alone and in larger doses for several months.

FIG. 7. A detailed view of the right upper lobe infiltration in *Case 5*. Within the lesion in the right second anterior interspace may be seen a crescentic radiolucency surrounding a smooth, rounded density. This proved to be a mycetoma when resected.

Comment: The entire seven-year clinical course of this elderly man would be compatible with chronic, indolent nocardiosis. Although the initial sputum culture was said to show *M. tuberculosis*, the details of identification are unknown. Because the patient did not tolerate combined tetracycline–sulfonamide therapy, suitable nocardiosis treatment has not been given and *N. asteroides* has been intermittently present in his sputum for eighteen months.

Case 7. J.W., a forty-three-year-old, Negro carwasher, with known glomerulonephritis and the nephrotic syndrome, was admitted to Wadsworth Veterans Hospital for the third time on October 24, 1958. The patient was first seen in July, 1957, when subacute glomerulonephritis was diagnosed by renal biopsy. His initial course was progressively downhill until prednisone administration produced a good diuresis. His second admission was in December of the same year for an exacerbation of the disease, which again responded to 60 mg. of prednisone daily. Physical examination on the third admission revealed an apparently middle-aged Negro in no distress. A blood pressure of 170 systolic, 110 diastolic in mm. of mercury, Grade I retinopathy, and moderate ankle edema were the only positive physical findings. Admission laboratory work revealed a hemoglobin of 11 gm. per 100 ml. and a leukocyte count of 13,000 per cu. mm. with a normal differential. The creatinine was 1.8 mg. per 100 ml. and the albumin and globulin were 1.7 and 3.3 gm. per 100 ml., respectively. The cholesterol was 710 mg. per 100 ml. and the patient was losing 10 gm. of protein in his urine daily. In addition, there were a few leukocytes and many granular casts in the urinary sediment.

The patient made no improvement on a conservative regimen, so was started on a course of adrenal corticosteroid therapy in various amounts in December, 1958. After two months of this therapy he developed pleuritic right lower chest pain, rusty sputum, and a right pleural effusion. His leukocyte count was 3,250 per cu. mm. Thoracentesis yielded a small amount of thick, yellow, purulent fluid which was negative on routine culture and smear. Three weeks later the patient had similar pain on the left with a pleural friction rub; however, roentgenographic examination was negative. Ten days later the pleuritic pain had increased and a roentgenogram then showed a pleural effusion. Several thoracenteses were done over the next three weeks and *N. asteroides* was cultured from the fluids obtained. Closed thoracotomy was performed with drainage of approximately 2,200 ml. of purulent fluid and the patient was placed on sulfadimethoxine therapy.

Two weeks after treatment was instituted, the patient developed symptoms of peritonitis, and a peritoneal tap yielded 2 ml. of purulent fluid which on smear showed gram-positive, filamentous organisms resembling Nocardia. Unfortunately, the culture was discarded. Over the next two months he received continuous steroid therapy, sulfadimethoxine for his nocardiosis, antimicrobials for sporadic bacterial infections, and drainage of his empyema cavity. He was dischaged in June, 1959, and since that time has had one additional admission for exacerbation of his nephrotic syndrome and glomerulonephritis. He has been maintained on a course of intermittent steroid therapy and there has been no recurrence of his nocardial infection up to the present time.

Comment: This man with histologically proved subacute glomerulonephritis required long-term administration of adrenocortical steroids to control his nephrotic syndrome. Coincident with prednisone administration, he developed nocardial pneumonia, empyema, and probable peritonitis. His infection was finally cured by thoracostomy and four months of sulfadimethoxine treatment.

Case 8. J.B., a five-year-old white boy, and his brother both had dysproteinemia with a demon-

strated failure to respond to antigenic stress and had suffered from repeated infections. The brother died in another hospital of disseminated aspergillosis. In December, 1957, the patient was given an empirical course of radiation to his chest, gamma globulin injections, and intermittent courses of antimicrobial drugs. In September, 1958, he developed cough and fever which became progressively worse and was associated with deterioration of his condition, although the chest roentgenogram was essentially clear. Large doses of chloramphenicol were given. He was admitted to the UCLA Medical Center for the sixth time on November 4, 1958. Physical examination revealed a thin, chronically ill child in acute respiratory distress. There were numerous rales throughout both lung fields. The heart was not enlarged. The liver was tender and down approximately 7 cm. below the costal margin. The urine was strongly positive for acetone. A blood count revealed a hemoglobin of 8.5 gm. per 100 ml. and 7,500 leukocytes with 83 per cent polymorphonuclear leukocytes. An electrocardiogram was suggestive of cor pulmonale and right ventricular strain. Chest roentgenograms showed massive bilateral pneumonia, and admission sputum culture grew *N. asteroides*. He was treated with erythromycin, novobiocin, sulfadiazine, and gamma globulin. He failed to respond and died one week after admission. Permission for autopsy was refused.

Comment: This case represents a clear example of nocardiosis in a patient with impaired host defenses. The numerous courses of antimicrobials the patient received probably played an important role in the origin of the nocardial infection.

Case 9. J.H., a sixty-seven-year-old, white male, entered Wadsworth Veterans Hospital for the first time on August 4, 1959. The admission history was incomplete, but it was learned that he had had a long history of emphysema and bronchiectasis and a stroke three years previously. He was hospitalized for a recent period of weakness and inability to care for himself. Physical examination revealed a chronically and acutely ill man with a blood pressure of 140 systolic, 68 diastolic in mm. of mercury, a pulse rate of 100 per minute, and a temperature of 39.5°C. The patient was in respiratory distress. Examination revealed signs of emphysema with rales and a friction rub over the left lateral and posterior lung fields. The heart was enlarged and the rhythm was grossly irregular. There was evidence of motor aphasia and a tremor of the right hand. His admission hemoglobin was 12 gm. per 100 ml., and the leukocyte count was 20,000 per cu. mm. with a shift to the left. A chest roentgenogram revealed patchy areas of consolidation throughout the left lower lung field and a smaller infiltrate at the right base. An electrocardiogram showed atrial fibrillation.

The patient was treated with digitalis and penicillin. Although his heart rate slowed, his fever did not lessen and he continued to run temperatures of 38° to 40°C. Roentgenographic and physical examination revealed a spread of his pulmonary infection. A week after admission there was evidence of cavitation in the left lung and fluid in the left pleural cavity. On August 9, 1959, a sputum smear showed a few acid-fast bacilli. Culture of this specimen did not grow tubercle bacilli, and blood plates were discarded after four days. Initial thoracentesis yielded only a small amount of straw-colored fluid which was sterile. Another thoracentesis revealed 200 ml. of yellowish fluid with 5,000 leukocytes, of which 82 per cent were neutrophils. A smear revealed occasional thin, gram-positive, branching, filamentous beaded organisms which could not be identified on an acid-fast smear. Unfortunately, this specimen also was cultured only on blood agar for four days.

The patient was treated for both tuberculosis (isoniazid and streptomycin) and nocardiosis (sulfadiazine) because of his critical condition. A closed thoracotomy and intubation were performed and he required a tracheotomy to facilitate control of his secretions. Within a few days there had been a temperature response and improvement in his roentgenogram. However, five days later, he again became febrile and his sputum showed numerous colonies of coagulase-positive *Staphylococcus aureus*. The patient responded to vancomycin but was found dead in bed sixteen days after admission without any known acute episode. Autopsy examination revealed pulmonary emphysema and bronchiectasis. There was a fibrinous exudate on the pleural surfaces of the left lung. Both lungs were involved with patchy areas of pneumonitis which were considerably more extensive on the left where there were cavitary changes. Microscopic examination revealed acute bronchopneumonia with abscess formation and pleuritis. In addition, the patient had arteriosclerotic heart disease and a duodenal ulcer.

Comment: Although cultural confirmation of nocardiosis was not obtained in this patient, it seems likely that the acid-fast organisms in his sputum and the gram-positive branching, beaded filaments in the pleural fluid were Nocardia. In addition, the clinical, roentgenographic, and pathologic pictures were all consistent with a nocardial infection. There was some response to the initial course of therapy, but the presence of severe underlying lung disease and superimposed staphylococcal pneumonia led to his death. This case also demonstrates the need for adequate cultures on appropriate media or the necessity of holding blood plates for at least thirty days if nocardiosis is considered.

Discussion

There is no pathognomonic clinical syndrome in nocardiosis. Any tissue may be involved,

TABLE 1
CLASSIFICATION OF NOCARDIOSIS

A. Acute
Focal acute abscess (any tissue)
Bronchopneumonia
With or without abscess
Locally invasive (pleura, chest wall)
B. Chronic
Chronic abscess (usually pulmonary)
Chronic fibronodular disease

which has led the writers to propose the classification shown in table 1. Even though the lung is most commonly affected, the extent and type of pulmonary disease varies considerably. All of the patients in the series had lung lesions; these included a chronic, solitary lung abscess; an acute, necrotizing pneumonia; and an indolent, progressive fibrosis resembling fibronodular tuberculosis. Five of the 9 patients had extension of the pulmonary suppuration to the pleura or chest wall, producing an empyema cavity, subcutaneous abscess, or bone involvement. One patient (*Case 3*) had numerous cutaneous fistulas from his intrathoracic abscesses. Despite the variability of thoracic nocardiosis, the extension of suppurative lung disease to the chest wall, with or without fistulas, strongly suggests nocardiosis. Dense fibrosis and multiple fistulas are more indicative of actinomycosis. To the writers' knowledge, pulmonary mycetoma ("fungus ball") has not been reported before in nocardiosis (*Case 5*, figure 7), although it is occasionally encountered in pulmonary mycoses, notably aspergillosis (37).

In addition to the local invasiveness shown in nocardiosis, the tendency for vascular involvement with metastatic abscess formation is demonstrated by *Cases 2*, *3*, and *4*. The unexplained propensity for metastatic nocardiosis to involve the central nervous system is shown by these cases. The patient of *Case 2* had a marked pleocytosis in the cerebrospinal fluid which is unusual in nocardial infection of the brain because the abscesses are usually deeply situated without a prominent meningeal reaction. Meningitis has been reported but is rare, and it was not possible to culture organisms from the cerebrospinal fluid in this case with clinical and autopsy evidence of leptomeningitis. In contradistinction to the findings in the cerebrospinal fluid, in which pleocytosis is unusual, serous exudates commonly contain many leukocytes which are characteristically polymorphonuclear. This may be a useful point in distinguishing nocardiosis and actinomycosis from the systemic mycoses and tuberculosis.

Although the brain was the most frequent site of extrathoracic nocardiosis in the present series, other lesions were found in the liver and kidneys in *Case 4* and in the spleen in *Case 3*. The frequency of disseminated nocardiosis indicates that at least transient septicemia must be fairly common; it is surprising, therefore, that positive blood cultures are rarely obtained, although they have been reported. None was found in any of the patients in the present series despite frequent cultures.

Although nocardiosis was not considered in any patient on admission, the diagnosis was subsequently made by recovery of the organism from sputum, thoracentesis fluid, or abscess drainage prior to death in 6 cases. In *Case 5* the disease was unsuspected until a resected lung specimen was cultured and examined microscopically. In *Cases 2* and *4* the diagnosis was established by recovery of the organism from autopsy material; however, in *Case 4* several antemortem sputum samples eventually yielded Nocardia after the patient's death from fulminating infection. In order to hasten and improve the diagnosis of nocardiosis, it is imperative that appropriate cultural techniques be used. Growth is readily obtained on blood and Sabouraud agar medium; antimicrobial drugs should not be added as they will inhibit the growth of Nocardia. Concentration methods used in preparing samples for tuberculosis culture will also inhibit nocardial growth, so specimens handled in this manner cannot be relied upon. Prolonged incubation of cultures is sometimes required.

Review of the literature and analysis of the present cases lead to the inescapable conclusion that the incidence of nocardiosis is increasing. Although the frequency of nocardial mastitis in cattle is also increasing, the organism is so ubiquitous in the environment that it is probably not a rising rate of bovine infection that accounts for the additional human cases. It is possible that an endogenous source of Nocardia is important as a source of disease. Rather than an increased prevalence or greater virulence of nocardial organisms explaining a higher incidence of the disease, it is the writers' contention that a changing status of the host renders him more susceptible to infection. There is probably

nothing unique in the ability of Nocardia to produce disease in this setting; it is one of many organisms, such as Aspergillus, Candida, and antimicrobial-resistant bacteria, that take advantage of alterations in the host's defenses and normal bacterial flora to thrive. Under most circumstances the body defense mechanisms and usual bacterial flora prevent the emergence of infection. How nocardiosis becomes established in the absence of underlying disease (*Cases 1, 2, 3, 5, 6,* and *9*) is unknown, but the incidence of this variety of infection does not seem to be changing. The appearance of nocardiosis as a secondary invader superimposed upon an underlying disorder probably accounts for the increased number of infections.

A defect in the immune processes may occur in many different diseases. There is also a convincing amount of evidence to support the notion that lowered immunity to infections results from the use of adrenocortical steroid drugs and many anti-neoplastic agents. When one or more of these factors exist, bacterial infection commonly occurs requiring prolonged antimicrobial therapy; it is in this setting that secondary infections can readily occur. The mandatory use of corticosteroids, anti-neoplastic drugs, and antimicrobials is widespread and increasing. A corollary of this practice is that such infections will also increase. This is an additional reason for avoiding the unnecessary or indiscriminate use of antimicrobials or steroids.

Three of the patients represent clear examples of the association of nocardiosis with grave underlying disorders: The patient of *Case 4* had pemphigus vulgaris requiring huge doses of prednisone, resulting in multiple toxic manifestations and disseminated nocardiosis. In *Case 7* nocardiosis developed during the treatment of subacute glomerulonephritis with long-term corticosteroid drugs. The patient of *Case 8* had received numerous antimicrobial drugs for infections resulting from a dysproteinemia, and he succumbed to nocardial bronchopneumonia. Several similar cases can be found in the recent medical literature. Hiddlestone (12) reported a patient with bronchial asthma requiring long-term prednisone administration who died from generalized nocardiosis with endocarditis and positive blood cultures. Eichold (13) described a physician who had pemphigus vulgaris; during treatment with large doses of hydrocortisone he developed nocardial lung abscesses which were successfully treated with sulfadiazine and sulfamerazine. Recently, Larsen and her associates (2) demonstrated nocardial infections in four patients with acute leukemia, chronic myelogenous leukemia, chronic lymphatic leukemia, and Hodgkins disease. All of these patients received corticosteroid therapy and numerous anti-neoplastic drugs. The nocardial infection was a contributory factor in the deaths of three of the four patients. The other patient was cured of his nocardiosis by surgery but died eighteen months later from leukemia. Two of the eight patients with fatal disease in the original report of pulmonary alveolar proteinosis (14) died with pulmonary nocardiosis. It is not clear whether or not these patients had received adrenal steroids; however, Larsen and associates conclude that fungous diseases associated with pulmonary proteinosis "may be abetted by prolonged use of antibiotics and adrenocorticoids." In a recent report of nocardiosis in children (20), one of the patients had the infection as a complication of a disordered immune mechanism very similar to that found in the present *Case 8*. Bianco and associates (11) briefly cited a young woman who died from *N. asteroides* peritonitis during treatment of lupus erythematosus with large amounts of cortisone. Finally, Seeliger and Lange (10) described a young woman who died of Hodgkin's disease and revealed at autopsy numerous subcutaneous abscesses of *Nocardia asteroides*. Although the abscesses were at injection sites, the infection was attributed to the "lowered general resistance" in Hodgkin's disease.

The prognosis of uncomplicated nocardiosis in the present series depended primarily upon the extent of the infection, when the disease was recognized, and the effectiveness of chemotherapy and surgical treatment. It is obvious that the prospects of any underlying disorder seriously affect the outcome of nocardiosis. Although the prognosis of patients with nocardiosis and coexisting disease is poor, it is not hopeless, as shown by *Case 7* and in reports by Eichold (13) and Larson, Diamond, and Collins (2).

The diagnosis of nocardiosis has always been difficult due to the variable manifestations of the disease; when the infection occurs with a multicentric systemic disorder, the clinical features are even more complex. Difficulties in distinguishing between tuberculosis, actinomycosis, and nocardiosis have troubled physicians for many years. There are clinical, roentgeno-

graphic, and bacteriologic similarities in these diseases which lead to problems in differentiation and confusion when they coexist. Expert bacteriologic assistance is necessary to distinguish the infections. The patients of *Cases 3* and *6* had pulmonary tuberculosis established by positive cultures prior to the onset of their nocardiosis. It is not believed that the associated tuberculosis altered the outcome in either patient except perhaps to delay slightly the diagnosis of nocardiosis in *Case 3*.

Awareness of the possibility of nocardiosis and obtaining appropriate cultures are the most important aspects favoring early diagnosis. Nocardial invasion should be considered especially when underlying circumstances enhance secondary infection. Vigorous chemotherapy and surgical treatment can eradicate the infection and should lead to a successful outcome in many patients.

Summary

Nocardiosis was once considered a clinical and laboratory curiosity. More complete bacteriologic studies on appropriate media, kept long enough to detect slow-growing colonies, have added to the number of cases recognized in the past decade. Although better means of detection have undoubtedly increased the apparent frequency of the disease, the incidence appears also to be rising due to a greater susceptibility of the human host. Nocardiosis is one of the infections that may occur when there has been an alteration in the host's defenses and normal bacterial flora; this predilection for infection may be part of a disease process or it may be induced by therapeutic measures (especially adrenocortical steroids).

Nine cases of nocardiosis are presented in addition to a review of fundamental aspects of bacteriology, pathology, and treatment. One of the most notable characteristics was the variety of disease encountered. The most common form was acute bronchopneumonia, often with empyema and/or chest wall extension. Three patients had generalized nocardiosis with brain abscesses. Two of the patients developed nocardiosis as a complication of high-dosage adrenocortical steroid therapy, and a third patient with dysproteinemia who had received long-term antimicrobials died from nocardial bronchopneumonia.

Requisite for improving survival in nocardiosis are awareness of the potentialities of the infection and adequate medical and surgical therapy. This is especially true when underlying circumstances enhance secondary infection.

Sumario

El Variable Aspecto de la Nocardiasis: Reseña y Presentación de Nueve Casos

La nocardiasis fué considerada una vez como curiosidad clínica y de laboratorio. Más completos estudios bacteriológicos en medios apropiados, continuados el tiempo suficiente para descubrir colonias de desarrollo lento, han acrecentado el número de los casos reconocidos en el último decenio. Aunque el perfeccionamiento de los medios de pesquisa ha aumentado indudablemente la aparente frecuencia de la enfermedad, también parece que se ha elevado la incidencia debido a la mayor susceptibilidad del huésped humano. La nocardiasis es una de las infecciones que puede presentarse cuando ha habido una alteración de las defensas y la flora bacteriana normal del huésped; esta predilección para la infección puede formar parte de un proceso patológico o ser provocada por las providencias terapéuticas (sobre todo los esteroides córticosuprarrenales).

Además de una reseña de la bacteriología, la anatomía patológica y el tratamiento, se presentan 9 casos de nocardiasis. Una de las características más notables fué la variación en la enfermedad encontrada. La forma más común fué bronconeumonía aguda, a menudo con empiema y/o difusión a la pared torácica. Tres enfermos tenían nocardiasis generalizada con abscesos cerebrales. Dos de los enfermos manifestaron nocardiasis como complicación de la esteroidocórticosuprarrenoterapia a altas dosis, y otro enfermo con disproteinemia que había recibido una larga serie de antimicrobianos falleció de bronconeumonía nocárdica.

Lo requerido para acrecentar la sobrevivencia en la nocardiasis es la comprensión de las potencialidades de la infección y la adecuada terapéutiva médica y quirúrgica. Esto es cierto en particular cuando las circunstancias subyacentes refuerzan la infección secundaria.

Resume

Le spectre changeant de la nocardiose: Une revue et une présentation de 9 cas

Il y eut un temps ou la nocardiose était considérée comme une curiosité clinique et de laboratoire. Des études bactériologiques plus complètes sur des milieux appropriés, conservés assez longtemps pour détecter des colonies poussant lentement, ont ajouté aux nombre de cas reconnus dans la dernière décade. Bien que de meilleurs moyens

de détection aient indubitablement accru la fréquence apparente de la maladie, sa rencontre semble être aussi en hausse grace à une susceptibilité plus grande de l'hôte humain. La nocardiose est une infection qui peut se produire quand il y a eu un changement dans les défenses de l'hôte et dans la flore bactérienne normale; cette prédilection pour l'infection peut être inclue dans le processus de la maladie, ou elle peut être suscitée par des mesures thérapeutiques (spécialement les stéroïdes adrenocorticaux).

Neuf cas de nocardiose sont présentés en plus d'une revue des aspects fondamentaux bactériologiques, pathologiques et thérapeutiques. Une des plus notoires caractéristiques fut la variété de maladie rencontrée. La forme la plus commune fut la bronchopneumonie aiguë, souvent avec empyème et extension à la paroi thoracique. Trois malades eurent une nocardiose généralisée avec abcés du cerveau. Deux des malades développèrent une nocardiose comme complication de hautes doses thérapeutiques de stéroïdes adrénocorticaux, et un troisième malade avec dysprotéinémie qui avait reçu des antibiotiques à long terme, mourut de bronchopneumonie à nocardia.

Ce qu'il faut pour améliorer la survie dans la nocardiose, est d'être averti des possibilités de l'infection et une thérapeutique médicale et chirurgicale adéquates. Ceci est particulièrement vrai lorsque des circonstances sous-jacentes exagèrent une infection secondaire.

Acknowledgment

The writers wish to thank Dr. Ruth Gordon, Rutgers Institute of Microbiology, for identifying the organisms from *Cases 3* and *5*.

Addendum

Since the preparation of this report another case of nocardiosis associated with pulmonary alveolar proteinosis has appeared (38). The writers also suggest that patients with proteinosis may be more susceptible to secondary infection with Nocardia.

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THE CONCEPT OF COLLAGEN DISEASES IN MEDICINE[1,2]

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(Received for publication August 4, 1960)

The topic of this address was suggested by the Program Committee and I have accepted it with some apprehension. While my co-workers and I (1) are responsible for the term and a somewhat vague concept behind it, I do not feel that we have contributed very much to its further elucidation. Yet great advances have been made by others in the nearly twenty years since the term was proposed as an heuristic idea which, if further explored, might have some importance for the comprehension of the nature and pathogenesis of certain puzzling maladies. I should like to present to you the evolution of observations and thoughts which have led to the concept and the sequence of investigations which mark the progress of our knowledge within the past two decades.

The term "collagen diseases" is generally used synonymously with that of "diseases of the connective tissue." I shall later in my presentation touch upon the reason we preferred the former designation, but I might say here that I do not object to the latter term if one is aware of the assumptions upon which both terms are predicated.

A systemic implication of the fibrous tissue was postulated in 1838 by Bouillaud (2) to account for the simultaneous involvement of the joints and of the heart in acute rheumatic fever; but this induction, important as it was for diagnosis and prognosis in clinical medicine, did not stimulate investigations of the pathogenesis of rheumatic fever. One can understand this failure if one considers the state of information about the connective tissue one hundred and twenty years ago. Nearly up until that date it had been regarded as a primordial tissue endowed with almost miraculous potencies of proliferation and unlimited differentiation. But a reaction had taken place in Bouillaud's time, and it had become an insignificant component of the animal body assigned a mere mechanical function to support the connection of organs and to fill the spaces between the nobler elements of the organism. For nearly eighty years the connective tissue was neglected in any scientific considerations of the pathogenesis of human disease, except for the ill-fated hypothesis of Virchow of the transcendent role of fibroblasts in new formations of all types. Subsequently, the dominance of Virchow's cellular pathology prevented investigators from paying attention to alterations of its intercellular component, and this ubiquitous constituent of the body remained a stepchild of scientific medicine. In the second decade of the twentieth century, Heinrich Schade (3) awakened interest in the physiologic role of the "connective tissue organ" for the control of water metabolism and the maintenance of ionic equilibrium. But it was Fritz Klinge (4) who, some thirty years ago, recognized widespread microscopic lesions in the intermediate parts of the connective tissue as a common morphologic denominator of a series of hitherto unconnected diseases. These alterations, commonly referred to as fibrinoid connective tissue damage or degeneration, had not escaped the attention of competent histopathologists before, foremost among them E. Neumann (5); but Klinge was the first who realized their significance for interpretation of the pathogenesis of certain etiologically puzzling diseases such as rheumatic fever, rheumatoid arthritis, periarteritis nodosa, dermatomyositis, malignant nephrosclerosis, and certain types of nephritis. He became convinced that these microscopic tissue changes provided a clue for etiology when he demonstrated the existence of similar lesions in the connective tissue of rabbits made hypersensitive by repeated injection of horse serum. The allergic theory to account for the etiology of rheumatic fever and, more generally, for other maladies with conspicuous fibrinoid connective tissue damage, was accepted by some of the leading pathologists of Germany and Scandinavia. The acceptance became even more universal when improvement in the technique of provoking hypersensitivity

[1] From the Cell Research Laboratory of the Mount Sinai Hospital, New York, New York.

[2] Lecture delivered at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 18, 1960.

(Rich, 6) made the experimental connective tissue and vascular alterations more conspicuous and reproducible. Bergstrand's publication (7) reflected the opinion that any malady should be regarded to have an allergic background if microscopic examination revealed fibrinoid connective-tissue damage. Accordingly, generalized scleroderma was included within the group of allergic diseases in 1938 (8).

It must be mentioned, however, that the hypersensitivity theory based upon morphologic similarity was not universally accepted, and that authoritative investigators pleaded for greater caution. Thus, Aschoff (9) maintained in 1935 "that it is open to doubt, if etiologically totally different diseases, though clinically similar, are designated as allergic for no other reason than that certain local histologic morbid products reveal a similarity with experimentally produced allergic inflammations in animals," and Opie (10) wrote in 1936, "The evidence assembled to show that allergy is essential to the production of certain diseases, for example rheumatic fever, is inconclusive." However, for the majority of investigators the evidence derived from animal experimentation seemed to prove fully the validity of allergic causation of a group of diseases which etiologically had been obscure. It was logical that further investigations should concern themselves mainly with the search for the antigens responsible for the immunologic abnormality, advocating a shift from structural to functional explorations, particularly in the field of rheumatic fever research. Indeed, that period witnessed important advances in the analysis of the antigenic properties of the hemolytic Streptococcus A in the search for the factor responsible for the disease; but an allergic mechanism was not proved unequivocally by animal experiment or immunologic examinations of patients.

In the course of clinical and pathologic investigations of disseminated lupus erythematosus, we (11) became aware of the prominence of fibrinoid connective-tissue damage in the histopathologic picture of the disease. In an etiologic interpretation of the tissue alterations we could have applied the attractive explanation of allergy which had found so much acceptance in that period. A critical consideration, however, of the clinical and morphologic observations caused us to question this hypothesis. On the one hand, the clinical and laboratory phenomena of disseminated lupus did not display those features which are commonly associated with diseases of an obvious allergic background, such as provocation by a specific agent, concomitant bronchial asthma, or urticaria and eosinophila in the peripheral blood. On the other hand, fibrinoid connective tissue and vascular damage can be observed in local sites as being evoked by factors having no relationship to allergy, such as ferment action (vicinity of pancreas necrosis) or rapid elevation of intra-arterial blood pressure (experimental hypertension). These were the reasons which caused us to caution against the etiologic generalization proposed by Klinge. We unreservedly agreed with him, however, that the systemic connective-tissue damage observed by him and by us in a series of etiologically obscure diseases was of utmost significance, and that continued investigation might reveal other clues for pathogenetic interpretation. We proposed, therefore, a reorientation of further investigations from "inordinate preoccupation with immediate cause" to closer analysis of the fundamental connective-tissue alterations. What we recommended was a strategic retreat from an overextended position of premature etiologic synthesis to an area fully clarified by basic structural and functional information. Such a retreat would permit renewed investigations of the factors which control alterations of the connective tissue, such as fibrinoid damage of its intercellular components. This was the thought which led us to propose a nonprejudicial term, referring only to the seat of the anatomic lesions. The choice of "collagen diseases" instead of the broader designation "connective-tissue diseases" was decided upon because our observations had shown that the intercellular components were conspicuously implicated, and not the cellular elements. It seemed to us that investigations of these maladies were a problem of intercellular and not cellular pathology. Furthermore, fibrinoid connective-tissue damage had been widely assumed at that time to be the result of a fundamental chemical alteration of the collagenous fibers, an assumption which also accounted for the choice of the term. Finally, it was not designed to delineate a well-defined disease entity, but should primarily reflect an heuristic idea which might promote a better comprehension of a group of puzzling maladies through a better understanding of the connective tissue. Accordingly, I have stressed in my

subsequent publications (12) mainly the conceptual but not the diagnostic connotation of the term.

If we survey the state of factual information about the connective tissue in 1941, we have to admit that it was not negligible and that the basic problems of its biology were already clearly perceived. If we view it today, we are impressed by the advances which have been achieved within less than two decades (for literature, see Jackson, 13). The ultrastructure of the collagen fiber has been ascertained with the aid of X-ray diffraction and the electron microscope; analysis of the chemical compound collagen has revealed its unique amino-acid constitution; physicochemical information derived from studies of the variety of soluble collagens has clarified the molecular structure of this protein and given insight into its synthesis. Important data have been accumulated on the various mucopolysaccharides of the ground substance and, recently, also on its proteinic components, and the investigations of the influence of enzymes and hormones have contributed much to a better understanding of the physiology of the connective tissue.

A detailed review of the amazing amount of research in the entire field would be not only impossible within a limited time, but also not profitable for the physician concerned with the problems of disease. But it is appropriate to ascertain what basic investigations have already contributed to a better comprehension of the mechanisms by which the maladies concerned are provoked, how much further information the physician can expect from the methods applied in the fundamental studies, how much light sophisticated connective-tissue research may throw on maladies originally not included in the group of collagen diseases, such as atherosclerosis, the heritable disorders of the connective tissue and, possibly, pulmonary emphysema.

A schematic diagram of the connective tissue reveals that it is composed of cells, fibers, and a homogenous matrix commonly referred to as ground substance. The principal fibrillar component is the collagenous fiber, and we attached great importance to a study of this structure. In fact, the choice of our term was greatly influenced by Neumann's assumption, which was widely though not universally accepted at that time, that fibrinoid connective-tissue damage reflects an alteration of its chemical constituent, the protein collagen, whereby it acquires the tinctorial qualities of fibrin. A more penetrating histopathologic, chemical, and physical analysis of pertinent tissues revealed subsequently that this proposition was incorrect and that the exclusive anatomic site of the fibrinoid substance was the interfibrillar ground substance and not the collagen fiber. This observation directed further research to the question of the nature of fibrinoid and the factors responsible for its precipitation (14).

The inquiry into the first of these problems was retarded by the fact that one was limited in the main to microscopic studies aided only by inadequate histochemical methods or by all too complicated techniques of enzymatic digestion. The results of these explorations were, therefore, equivocal. A powerful technique for the identification of proteins in tissues is the recently discovered fluorescent antibody method of Coons and Kaplan (15). An antiserum prepared by injection of a rabbit with various isolated plasma proteins is conjugated with a compound, fluorescin isothiocyanate, and brought in direct contact with the tissue section to be analyzed. If the tissue contains the homologous antigen, a fluorescent precipitate is formed with the antibody of the rabbit serum. This technique has recently been applied in the identification of fibrinoid substance in rheumatic fever, systemic lupus erythematosus, rheumatoid arthritis, periarteritis nodosa, and experimental serum sickness (16–18). Gamma globulins have been found by all investigators collectively, although some have also recognized a prominent quantity of fibrin (19).

The presence of gamma globulins in the fibrinoid of the "collagen diseases" has been accepted by some as adequate evidence for the operation of an immunopathologic mechanism in these maladies, since gamma globulin was equated with antibody. This, of course, is still a controversial assumption. Critical investigators, foremost among them Dixon and his associates (18), caution against such far-reaching conclusions by pointing to certain limitations of the pertinent observations. They illustrate that fluorescent deposits of gamma globulins in a tissue lesion need not necessarily reflect an antibody-antigen reaction but may merely be part of the nonspecific inflammatory exudate. The complex identity of the fluorescent deposits can be ascertained in animal experiments in which the anti-

gen is definitely known, but not in human lesions where the provoking antigen is unknown. Further painstaking investigations of human diseases are needed before the allergic etiology can be accepted as unequivocally proved. I shall return to the question again when discussing the hypothesis of auto-immunization in systemic lupus erythematosus and rheumatoid arthritis.

While the investigations of Altshuler and Angevine (20) and my own studies, preceded by those of Klinge, have shown that the collagen fibers are not primarily implicated in fibrinoid connective-tissue damage—"the hallmark of collagen diseases"—their further investigations are still important in clarifying the pathology of these and other etiologically obscure maladies. It is fortunate that the renaissance of medical concern with the connective tissue coincided with an awakening of interest among biophysicists in the ultrastructure of the collagen fiber. The discovery of characteristic electron-microscopic and X-ray diffraction features made its identification unequivocal and was the start of profound research of the molecular biology of collagen. This led to the resumption of investigations of basic connective-tissue problems which had intrigued the great histologists of the nineteenth century since the days of Theodore Schwann. Foremost among these questions is that of fibrillogenesis, the exact origin of the fibers, and its relationship to the amorphous ground substance.

Summarizing the numerous reports of the preceding century (21), it can be said that, in 1941, the majority of competent investigators believed that collagen fibers were manufactured by the fibroblast and subsequently laid down in the ground substance, but an equally competent minority believed in extracellular origin with more or less auxiliary activity of connective-tissue cells. In spite of most painstaking investigations of embryology, comparative anatomy and, finally, the application of tissue cultures, the problem of fibrillogenesis could not be answered unequivocally and an impasse seemed to have been reached. Electron-microscopic investigations and physicochemical studies of the molecular constitution of collagen gave greater assurance to the accuracy of observations because the periodic band structure of the fibers served as a fingerprint for its ready identification in a variety of analytic studies. It is at present agreed that the fibroblast synthesizes the monomeric macromolecules of collagen, tropocollagen, or procollagen (22); fibrillar polymerization with formation of the primordial fibril occurs possibly at its plasma membrane (23), but is fully completed in the extracellular space. It is assumed that the macromolecules are freely dispersed within the ground substance, and that they precipitate upon the primordial fibril when it is separated from the surface of the fibroblast.

The knowledge of the ultrastructure of the collagen fiber, of the chemical constitution and physicochemical behavior of the collagen molecule, has been greatly advanced within the past decade, but not too much of this information has as yet been applied for the explanation of morbid states affecting the connective tissue. F. O. Schmitt, one of the foremost pioneers in collagen research at the molecular level, indicated in 1959 (24) that pathologic processes may have their origin in interactions of collagen macromolecules with others of their own or of another kind. This all too general assertion was not greatly amplified by his answer to the question of a classical pathologist—whether it was worth while to study pathologic material with the electron microscope. Dr. Schmitt's remark that, "a collagen fibril that looks a little different or stains differently cannot with certainty be regarded as pathological" (24), was certainly not encouraging. In view of the complexity of the problems of molecular biology of normal collagen—and, it might be added, of the normal ground substance—attested to by the cautious advance of fundamental research, the medical investigator must control his impatience. But I believe he should point to the problems of pathologic states of the connective tissue, and indicate that their exploration may also serve the comprehension of the normal.

Some recent investigations of morbid conditions may illustrate the potentiality of research with the refined tools of modern biology. The partial solubility of collagen in weak acetic-acid solutions was first recognized by Zachariades in 1900 (25). His studies were resumed by Nageotte in 1927 (26) and significantly expanded (27) by the demonstration that collagen fibers can be reconstituted from the fiber-free solutions by neutralization with alkali or salts. Nageotte's observations became, within the last decade, the starting point for the study of molecular structure and metabolism of this protein. A variety of other solvents were tested for ex-

traction of fresh connective tissue, such as 0.1M citrate, alkali and, finally, cold neutral salt solutions at hypertonic and physiologic ionic strength. From all solutions, collagen fibers could be precipitated by neutralization or temperature adjustment to 37°C. Quantitative determinations of the amounts of extractable collagen, measured by its hydroxyproline content in growing and growth-inhibited rats, and studies of the incorporation of radioactive amino acids provided strong evidence indicating that the collagens extracted by the different solvents differ in the age of their synthesis. Gross (28) concludes from carefully controlled studies that the alkaline– and neutral salt–extractable molecules are the most recently synthesized collagens, which account for about 10 per cent of the total dermal collagen in actively growing eight-day-old guinea pigs.

With all the information gained from the basic investigations of the biology of normal connective tissue, the time seems to have arrived to apply it in the study of pathologic states in which the formation of this protein is affected. Since the classical studies of Wolbach and Howe (29), it has been known that collagen fiber formation is arrested in scurvy. Orekhowitch and Shpikiter (30) found the citrate-extractable component sharply decreased in scorbutic guinea pigs, and Gross (28) observed a similar deficiency of the salt-soluble partition. These observations point to an arrest of synthesis of the precursors of the normal collagen fibers. A different disturbance of collagen metabolism was discovered by Levene and Gross (31) in their studies of the connective-tissue abnormality in lathyrism. The development of skeletal malformation (kyphoscoliosis) and rupture of the aorta can be induced by the toxic factor of *Lathyrus odoratus* in experimental animals. One of the dramatic features of the animal treated with β-aminopropionitrile, a purified potent lathyrogenic factor, is the loss of tensile strength of collagen fibers. In carefully controlled experiments on the developing chick embryo injected via the chorionic alantoid membrane with the chemical compound, Levene and Gross found a striking increase in fragility measured by the time required to separate the head from the body under a constant stretching load. The evident defect in tensile strength is associated with an increased extractability of the collagen fibers with cold hypertonic salt solution which can be made visual by light and electron-microscopic studies (32). Dr. Van Den Hoof and his associates believe that the alteration of the fibers is the result of a disruption of the intermolecular crosslinkings of the collagen which are strong in normally insoluble fibers.

These investigations seemed to me to be of more than academic interest. They indicate that the same techniques could be successfully applied to the study of human morbid states in which the clinical and pathologic-anatomic picture points to increased fragility of the connective-tissue fibers, such as in Marfan's disease. It is interesting that increased excretion of hydroxyproline, the essential amino acid of collagen, has been observed in the urine of such patients (33).

Conversely, there are maladies which are characterized by an inordinate increase of collagen fibers, and this suggests a possible decrease in their catabolism. Foremost among them is generalized scleroderma, but it is evident also in the chronic cardiac lesions of rheumatic fever, in the joint deformities of rheumatoid arthritis and, though only locally, in the periarterial splenic fibrosis in systemic lupus erythematosus. A careful analysis with the methodology of modern molecular biology might throw some light upon the factors responsible for the unbalanced formation of collagen fibers.

My survey of the biology of normal connective tissue has so far been concerned mainly with the collagenous component, and the amorphous ground substance has only been mentioned when discussing the role it plays as the anatomic site of fibrinoid connective-tissue damage. However, these investigations have not been less exhaustive than those of collagen. Several fundamental facts have been established within the past two decades. The chemical constitution of the acid mucopolysaccharides which form a prominent portion has been elucidated by Karl Meyer (34) and his school, and their metabolism and biosynthesis is being successfully pursued by Dorfman and his associates (for a review of the subject, see Dorfman, 35). It has been ascertained by tissue culture that the fibroblast is capable of manufacturing mucopolysaccharides (36), but the exact site and modality of synthesizing the eight different carbohydrate compounds which have been isolated by Meyer is still under investigation. The nature and origin of the proteinic component of the normal

ground substance has attracted less attention than that of the mucopolysaccharides. Histopathologic observations, however, indicate that abnormal proteins, such as amyloid, deposited within the ground substance might be derived from abnormal plasma proteins. Gueft and Laufer (37), in our laboratory at Mt. Sinai, have demonstrated that the fibrinoid substance in lupus erythematosus contains nucleoprotein, and that a histochemically identical substance is found within blood vessels. The studies with fluorescent antibodies, referred to before, also illustrate that the fibrinoid substance in collagen diseases includes plasma gamma globulins. These observations of pathology suggest that a transfer of protein from the blood into the connective-tissue ground substance may also occur under normal circumstances. Gitlin and associates (38) demonstrated with the fluorescent antibody method the presence of plasma proteins between the connective-tissue fibers, and Ludwig and Levin (39) in our laboratories found serum albumin and globulin in the purified mucin of synovial fluid and umbilical cord by paper electrophoresis. A recent report of Neuberger (40) that the skin and tendon of the rat and the rabbit contain a considerable proportion of plasma proteins identified by rigid physicochemical and immunologic tests is in agreement with the previous conclusions. The influence of enzymes and hormones on the plasticity of the ground substance is evident, but a detailed discussion exceeds the limits of this presentation.

It is obvious from microscopic examination of tissues derived from collagen diseases that alterations of the ground substance, such as fibrinoid connective-tissue damage and a striking accentuation of metachromasia indicating some change of the mucopolysaccharides, play a major part in their pathology and deserve the attention of fundamental investigations. How rewarding the planned cooperation can be between exact clinical observation and penetrating laboratory investigation has recently been illustrated by reports on the pathogenesis of Hurler's disease, or gargoylism, one of the heritable diseases of the connective tissue. Meyer and his associates (41) and, independently, Dorfman and Lorincz (42) have discovered abnormal amounts of the acid mucopolysaccharides in the urine of patients, and their storage in mesenchymal cells has also been demonstrated.

The foregoing discussion was intended to show how knowledge of the biology of normal connective tissue might advance understanding of its abnormal behavior in pathologic states. The strategy of medical research, however, calls for flexibility of attack and the utilization of all possible leads originating in physiologic research as well as in accidental observations of pathology. The discovery of the hematoxylin-stained bodies in tissues of patients with systemic lupus erythematosus by Louis Gross (43) and Ginzler and Fox (44) in 1932 and 1940, respectively, was an observation of far-reaching significance that was, however, not fully comprehended at that time. The recognition of the L.E. cell in bone marrow and blood by Hargraves and associates (45) and Sundberg and Lick (46), the histochemical analysis of the hematoxylin bodies by my co-workers and me (47), and their identification with the L.E. cells by Lee and associates (48) in our laboratories opened a new avenue for investigation of this disease. These observations were essentially of morphologic character, but seemed not to be correlated with the already known connective-tissue alterations. The discovery of the L.E. factor by Haserick and co-workers (49) as a component of the gamma-globulin fraction of the blood serum in lupus patients introduced an entirely new approach to the understanding of the pathogenesis of this disease. The nature of the interaction of the factor with the cell nuclei suggested that it was of antibody-antigen character, an idea which was supported by its residence within the gamma-globulin fraction. The mechanism of action of the L.E. serum factor upon nuclei of mesenchymal cells and, in particular, upon leukocytes became subsequently the object of ingenious investigations. By histochemical methods far surpassing our first explorations, Godman and Deitch (50–52), demonstrated a profound alteration and increase of the protein constituents of the cell nuclei. Miescher (53) observed by absorption studies an interaction between L.E. factor and the isolated chemical components of the nucleus, and the group headed by Kunkel and Holman at the Rockefeller Institute (54, 55) demonstrated that the various constituents of the cell nuclei fix complement if brought into contact with L.E. factor.

The complement-fixation tests strongly indicate that the L.E. serum contains a number of antibodies, elicited by the disease, against various fractions of nuclei. In the light of this hy-

pothesis, the old observation of false-positive Wassermann reactions so frequent in lupus erythematosus can be better understood. The significance of these immunologic revelations for the problem of the collagen diseases was deepened when clinical and laboratory studies showed a more than accidental relationship between systemic lupus erythematosus and rheumatoid arthritis indicated by the high percentage of positive L.E. tests in patients with unequivocal rheumatoid arthritis. Special studies referred to by Kunkel (54) disclosed a high incidence of rheumatoid arthritis in families of patients with lupus erythematosus.

These observations of the very last years presented by most careful investigators permit the cautious statement that two maladies of the group of collagen diseases reveal evidence of the presence of auto-antibodies, and that these findings suggest a state of altered immune mechanism which may be genetically determined. These disclosures promise a better insight into the pathogenesis of the collagen diseases if expanded by further careful clinical and laboratory studies.

* * *

Ladies and gentlemen, I have reached the end of my survey of the present state of the concept of collagen diseases in medicine. But we certainly are not at the end of the road. The new disclosures of the past twenty years have raised questions which provoke the curiosity of the physician and the ingenuity of the biologist. But we can admit, I believe, that the original idea with all its much lamented ambiguity has been of some value. It has focused attention upon the role of the connective tissue in human disease, a role which exceeds the confines of the group of puzzling maladies with which we were concerned originally. Two hundred years ago, Albrecht Von Haller (56) wrote in reference to the tela cellulosa, today's connective tissue, "that it is evident what a considerable part of the human body consists of matter which for so many centuries has been regarded as rubbish." These prophetic words are an encouragement and a challenge for us to continue investigations of the collagenous tissue with every method available.

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THE EFFECT OF INTERMITTENT POSITIVE PRESSURE BREATHING AND OF BRONCHODILATOR DRUGS ON ALVEOLAR NITROGEN CLEARANCE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY EMPHYSEMA[1, 2]

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(Received for publication May 16, 1960)

Introduction

In pulmonary emphysema the resting end-expiratory lung volume (functional residual capacity) is enlarged, with distortion of alveolar and bronchiolar anatomy. Inspired gas is distributed unevenly in this system, resulting in alveolar hypoventilation of increasing magnitude as the disease progresses. Expired gas encounters increased airway resistance, especially on effort. A commonly used treatment for this condition is intermittent positive pressure breathing (IPPB) with aerosolized bronchodilator drugs. Proponents of this therapy claim that it reduces airway resistance by relief of existing bronchospasm and mobilization of secretions; that it produces hyperventilation with minimal effort on the patient's part, which results in better alveolar ventilation, possibly with flushing out of previously poorly ventilated spaces; and that the whole process results in elevation of oxygen saturation and reduction of carbon dioxide content of the arterial blood (1–6). Those less impressed with the benefits of such treatment report favorable effects with simple nebulization of the same bronchodilator drugs without IPPB (7–11), thus agreeing at least that bronchial narrowing which is reversible to a small degree is a feature of emphysema.

Analysis of pulmonary nitrogen clearance curves during oxygen breathing in emphysema demonstrates the marked degree of uneven alveolar ventilation which characterizes this disease (12–16). The effect of hyperventilation on such clearance curves has not been reported extensively. It was the purpose of this study to determine the effect of intermittent positive pressure breathing of oxygen, with and without inhalation of isoproterenol hydrochloride aerosol, on nitrogen washout *during* the hyperventilation procedure. Nitrogen clearance during oxygen breathing before and after intravenous administration of aminophylline was also studied. Obviously, during hyperventilation produced by intermittent positive pressure breathing (IPPB), nitrogen washout will be more rapid than during eupneic oxygen breathing at ambient pressure (APB) since greater volumes of oxygen pass through the lungs in a given time. The questions that the present writers have attempted to answer are whether more nitrogen clearance occurred for equivalent volumes of oxygen breathed during IPPB with or without isoproterenol than during APB, and whether aminophylline facilitated nitrogen clearance. An affirmative answer would signify more efficient alveolar ventilation as a result of the treatment.[3]

Materials and Methods

Twelve male patients, hospitalized because of emphysema, were selected for study on the basis of clinical judgment, roentgenographic criteria, and previous pulmonary function measurements. Each was familiar with intermittent positive pressure breathing treatment and with procedures in this laboratory. The severity of the patients' disability was graded clinically according to a classification in use in the laboratory which describes the limitation of exercise tolerance due to dyspnea. None of these patients was in congestive heart failure at the time of study. Identifying characteristics are listed in table 1.

The following laboratory procedures were per-

[1] From the Veterans Administration Hospital, San Fernando, California; and the departments of Physiology and Medicine, School of Medicine, University of California, Los Angeles, California.

[2] Presented before the Medical Session, as part of *Section 4A*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 17, 1960.

[3] In speaking of efficiency of alveolar ventilation, this paper refers to the criteria of reduction of end-tidal nitrogen concentration and to accumulation of expired nitrogen in a collecting chamber during equivalent volumes of oxygen breathing rather than to rapidity of nitrogen clearance or to calculations of actual effective tidal volumes (tidal volume minus dead space volume).

TABLE 1

IDENTIFYING CHARACTERISTICS OF PATIENTS IN THIS STUDY

Patient Number	Age	Height	Weight	BSA*	Grade of Disability†	Asthma Past or Present	Complications
	years	*inches*	*pounds*	M^2			
1	58	67	156	1.82	III	0	0
2	58	71	141	1.82	III	0	Cor pulmonale
3	36	70	160	1.91	III	+	Bronchiectasis, cor pulmonale
4	48	73	148	1.89	III	0	0
5	63	68	142	1.88	IV	0	Cor pulmonale
6	60	72	150	1.88	III	0	Hypothyroidism
7	64	65	108	1.52	III	0	Cor pulmonale
8	62	70	120	1.68	III	0	0
9	63	71	123	1.72	III	0	0
10	60	70	127	1.72	IV	0	Cor pulmonale
11	67	72	158	1.93	IV	0	Cor pulmonale
12	49	65	154	1.77	III	+	0

* BSA = Body surface area in square meters.

† Grade of disability based on a clinical classification of dyspnea ranging from I to V in progressive severity.

III = exercise tolerance limited by dyspnea to walking one or two blocks; unable to climb stairs.

IV = unable to leave home or hospital ward because of dyspnea, but not bedfast.

TABLE 2

SCREENING VENTILATORY TESTS

	EVC	1.0 VC	3.0 VC	MEFR	MMEF	MBC
	L.	*L.*	*L.*	*L./min.*	*L./min.*	*L./min.*
Before intermittent positive pressure breathing and isoproterenol						
Mean	2.94	0.81	1.54	50	27	40
Range	1.80–4.43	0.38–1.90	0.81–2.93	13–179	12–95	21–72
After intermittent positive pressure breathing and isoproterenol						
Mean	3.58	1.15	2.02	77	35	59
Range	2.27–4.62	0.75–2.12	1.34–3.15	35–202	16–75	42–76

EVC = expiratory vital capacity in liters at body temperature and pressure, saturated with water vapor (BTPS)

1.0 VC = one-second vital capacity in liters (BTPS)

3.0 VC = three-second vital capacity in liters (BTPS)

MEFR = maximal expiratory flow rate, in liters per minute (BTPS)

MMEF = maximal midexpiratory flow rate, in liters per minute (BTPS)

MBC = maximal breathing capacity (BTPS)

formed: On the first day simple ventilatory tests were made before and immediately after a ten- to fifteen-minute period of IPPB with isoproterenol, using a Bennett IPPB apparatus.[4] The tests, made with a spirometer equipped with a kymograph moving at the rate of 32 mm. per second, are listed in table 2. The following day the nitrogen washout measurements were made after omission of all bronchodilator treatment and smoking since the previous night. Each subject was in the seated posture and breathed room air through two channels of a four-way valve from a balloon-in-bottle system. At the end of a normal expiration (monitored by a spirometer), the subject was switched to the other two channels of the respiratory valve, now inspiring oxygen from a Douglas bag and expiring into a kymograph-equipped Tissot spirometer (of 120-liter capacity for the first 6 subjects and of 350-liter capacity for the latter 6 subjects). The spirometer had been flushed previously with oxygen from the same tank which supplied the patient during the washout procedure. One nitrogen

[4] Donated for this study by Bennett Respiration Products, Inc., Los Angeles, California.

TABLE 3

VOLUME OF NITROGEN PASSING FROM EXTRAPULMONARY TISSUES INTO ALVEOLI DURING PROLONGED OXYGEN BREATHING

Duration (minutes)	2	4	8	12	16	20	25	30
Mean V_{N_2} (ml.)	94	154	237	322	374	434	486	543
Mean V_{N_2}/kg. (ml.)	1.22	2.01	3.09	4.20	4.88	5.66	6.34	7.08

Values based on findings in 10 normal males, ranging in age from 33 to 57 years (mean 45.7) and in weight from 67.5 to 90.0 kg. (mean 76.7)

V_{N_2} = Nitrogen volume corrected to 0°C. 760 mm. of mercury, dry (STPD), based on Van Slyke analysis

meter[5] automatically measured the nitrogen concentration of each breath at the mouthpiece, while a second nitrogen meter continuously sampled the expired gas mixture in the Tissot spirometer, in which a mixing fan operated. Recording of the nitrogen meter signals was made on a two-channel Sanborn direct-writing oscillograph. In the first 6 subjects oxygen breathing continued until the total expired volume equaled approximately 100 liters, while in the latter 6 subjects the test was carried on until approximately 200 to 250 liters of expired gas had been accumulated. This procedure constituted a nitrogen washout during eupneic ambient pressure breathing (APB).

After an intermission period of air breathing for thirty minutes for the first 6 patients, a similar procedure was carried out during IPPB with oxygen in the course of which a 1:200 solution of isoproterenol was aerosolized at the mouthpiece (IPPB with isoproterenol). The pressure used was 20 cm. of water, and the subject was allowed to choose his own respiratory rate. The discharge system of the IPPB apparatus was enclosed in an airtight chamber which emptied into the Tissot spirometer. The last 6 patients, after air-breathing periods of one hour following the APB procedure, were then subjected to intermittent positive pressure oxygen breathing without isoproterenol, and after a further rest of one hour they underwent the IPPB with isoproterenol procedure. Five of these 6 patients, at a subsequent or previous session, had APB washouts performed before and after administration of 0.5 gm. of aminophylline intravenously. For each patient the total expired gas volume was the same for APB and for IPPB with and without isoproterenol procedures, and for the pre- and post-aminophylline trials.

The total collected spirometer gas was analyzed for nitrogen by Van Slyke manometric methods at the end of each run as a check on the accuracy of the nitrogen meter readings. The nitrogen meters were calibrated before and after each run by their response to a series of mixtures of nitrogen-in-oxygen of known concentration, saturated with water vapor. The mean deviation from Van Slyke analysis of the nitrogen meter which sampled the spirometer was plus 0.16 per cent nitrogen, with a standard deviation of 0.13 per cent. The mean error of the mouthpiece-sampling nitrogen meter was plus 0.10 per cent nitrogen. No correction was made for the presence of argon and other trace gases, which, in the Van Slyke analysis, are included in the nitrogen fraction.

Calculations of functional residual capacity were made by conventional methods (17). All lung volumes were corrected to body temperature (BTPS). End-tidal nitrogen was considered alveolar. In the computations of nitrogen accumulated in the spirometer at various intervals during the washouts, correction was made for the lag of appearance of nitrogen from the expired gas which had to pass through the tubing leading from the subject to the Tissot spirometer. The functional residual capacity values were corrected for traces of nitrogen in the inspired oxygen, apparatus dead space, switching errors at the moment of turning the subject from air breathing to oxygen breathing, and diffusion of nitrogen from the blood into the pulmonary air spaces. This last correction was based on measurements in this laboratory of nitrogen collected from 10 normal persons during thirty minutes of oxygen breathing after discarding all of the expired gas from an initial period of hyperventilation with oxygen which rapidly reduced alveolar nitrogen concentration to 1 per cent (table 3).

The nitrogen washout data were utilized in two ways. The data from the mouthpiece-sampling nitrogen meter were correlated with the volume of each breath to make a curve relating "alveolar" (actually end-tidal) nitrogen concentration ($F_{A_{N_2}}$) to expired volume (V_E), as previously described (18) (figures 1, 2). In the other method, the data from the spirometer-sampling nitrogen meter were used to construct a curve relating nitrogen accumulated in the spirometer (V_{N_2}) to expired volume (figure 3).

In the first 6 patients studied, arterial blood samples were taken anaerobically during each procedure for pH determination and for oxygen and carbon dioxide measurement by Van Slyke methods, and continuous monitoring of the carbon dioxide in the end-tidal air was done with an infrared carbon dioxide meter.

Statistical methods used were the Kruskal-Wallis one-way analysis of variance for comparison of three samples and the Mann-Whitney *U* test for differences between two samples (19).

RESULTS

The markedly reduced gross ventilatory capacity of these patients and the small degree of absolute improvement after intermittent positive pressure breathing with isoproterenol are shown in table 2.

[5] Manufactured by the Waters Corporation, Rochester, Minnesota.

The degree of hyperventilation achieved during IPPB and IPPB with isoproterenol is revealed in table 4. The mean tidal volume for all 12 patients during eupneic oxygen breathing at ambient pressure was 0.79 liters. This increased to 1.10 liters during IPPB and to 1.69 liters during IPPB with isoproterenol. The greater tidal volume resulting from IPPB with isoproterenol was significantly different from that manifested during IPPB ($P = 0.003$), as well as twice as large as during APB. This indicates that the inhaled isoproterenol decreased airway resistance by increasing bronchial caliber, either as a consequence of relief of bronchospasm or of shrinking of turgid bronchial mucosa.

It will be recalled that in patients *Nos. 1–6* the sequence of procedure was APB followed by IPPB with isoproterenol, while in patients *Nos. 7–12* the sequence was APB, IPPB, and IPPB with isoproterenol. Comparison of the magnitudes of the tidal volumes during IPPB with isoproterenol in both groups disclosed no significant differences ($P = 0.29$). Similarly, the differences between the APB tidal volumes and the IPPB with isoproterenol tidal volumes for the two groups were not significant ($P = 0.80$). It is concluded, therefore, that the prior IPPB trial in the last 6 patients had no effect on the tidal volume of the subsequent IPPB plus isoproterenol procedure.

The enormous sizes of the functional residual capacities, residual volumes, total capacities, and the large ratios of residual volume to total capacity are shown in table 4. Changes in functional residual capacity as a result of IPPB with or without isoproterenol were not consistent in direction. It should be pointed out that the measured size of the functional residual capacity depends on two factors (assuming constancy of tidal volume). One is the uniformity of alveolar

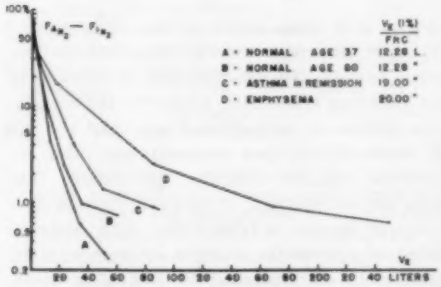

FIG. 1. Representative nitrogen washout curves for 2 normal subjects, a patient with asthma, and a patient with emphysema, during ambient pressure oxygen breathing. In this and subsequent figures, $F_{AN_2} - F_{IN_2}$ = end-tidal ("alveolar") nitrogen corrected for nitrogen in the inspired oxygen supply; V_E = total amount of oxygen breathed, or total expired ventilation; V_E(1 per cent)/FRC = total ventilation required to lower F_{AN_2} to 1 per cent per liter of functional residual capacity.

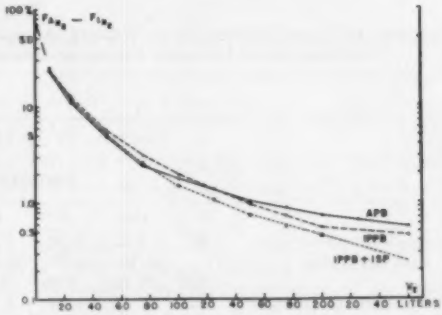

FIG. 2. Composite nitrogen washout curves for patients *Nos. 7–12*, based on average values of table 6. The curves represent pulmonary nitrogen displacement during oxygen breathing at ambient pressure (APB), intermittent positive pressure breathing without isoproterenol (IPPB), and intermittent positive pressure breathing with isoproterenol (IPPB with ISP).

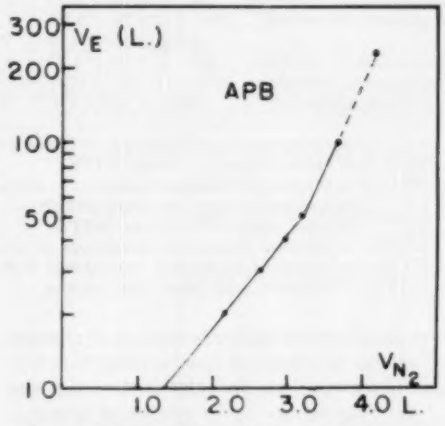

FIG. 3. Accumulation of nitrogen in spirometer as ventilation with oxygen progresses, based on mean values for ambient pressure oxygen breathing in table 5. Data are from patients *Nos. 7–12*. Seventy-eight per cent of the total nitrogen displaced from the lungs has been collected at the inflection point of 50 liters of ventilation.

TABLE 4

CHANGES IN TIDAL VOLUME, LUNG VOLUME, ARTERIAL pH AND CO_2 AND END-TIDAL CO_2 AS RESULT OF INTERMITTENT POSITIVE PRESSURE BREATHING WITH OR WITHOUT ISOPROTERENOL

Procedure	Mean Values and Ranges									
	V_E	Duration	V_T	FRC	RV	TC	RV/TC	pH	P_aCO_2	P_ACO_2
Patients Nos. 1–6										
	L.	*Min.*	*L.*	*L.*	*L.*	*L.*	*per cent*		*(mm.Hg)*	*(mm.Hg)*
Eupneic ambient pressure breathing	97 (92–100)	8.97 (6.10–11.55)	0.96 (0.72–1.30)	5.46 (4.49–6.97)	4.79 (4.05–6.86)	7.39 (6.30–8.26)	64.6 (51.8–83.1)	7.395 (7.35–7.42)	43.7 (37.5–51.5)	33.9 (28.0–46.7)
Intermittent positive pressure breathing and isoproterenol	97 (92–103)	4.46 (2.70–5.81)	1.87 (1.22–2.64)	5.19 (3.85–6.67)	4.44 (3.31–6.43)	7.28 (6.02–8.39)	60.4 (53.6–80.2)	7.515 (7.47–7.59)	29.9 (21.3–37.9)	23.8 (15.5–29.2)
Patients Nos. 7–12										
Eupneic ambient pressure breathing	230 (131–253)	21.51 (16.40–30.78)	0.67 (0.53–0.83)	6.47 (5.68–7.77)	5.51 (4.52–6.80)	7.93 (7.17–9.29)	69.4 (62.5–76.2)	—	—	—
Intermittent positive pressure breathing	237 (201–252)	15.00 (12.52–15.99)	1.11 (0.93–1.43)	6.24 (5.22–7.27)	5.44 (4.77–6.10)	7.71 (6.72–8.64)	70.7 (61.9–78.7)	—	—	—
Intermittent positive pressure breathing and isoproterenol	238 (212–253)	9.73 (6.32–12.30)	1.53 (1.20–2.14)	6.34 (5.18–7.31)	5.47 (4.75–6.41)	7.90 (6.99–8.97)	69.4 (62.1–75.9)	—	—	—

V_E = total expired gas volume, in liters, at body temperature and pressure, saturated (BTPS)
V_T = tidal volume, in liters (BTPS)
FRC = functional residual capacity, in liters (BTPS)
RV = residual volume, in liters (BTPS)
TC = total capacity, in liters (BTPS)
RV/TC = residual volume as percentage of total capacity
P_aCO_2 = arterial CO_2 tension (calculated from Henderson-Hasselbalch equation)
P_ACO_2 = alveolar (end-tidal) CO_2 tension

ventilation, which limits the amount of nitrogen which can be displaced from a given lung volume in a given time. The other is the actual size of the lung volume to be cleared of nitrogen, which is the volume at the moment the subject is switched from air breathing to oxygen breathing. While an effort was made to have the patient achieve a steady resting expiratory level before starting each washout, there was no assurance that this level was the same prior to each procedure. In this connection it is well to realize that the end-expiratory level may change during the washout procedure from that established before beginning the washout, especially during hyperventilation. Such variations in functional residual capacity will affect the slopes of the washout curves in a lung model (20).

While there were individual variations in the nitrogen clearance patterns, it became expedient for purposes of statistical handling of the data to deal with mean values of the entire group.* Nitrogen clearance will be described in two ways, namely, the accumulation of nitrogen in the collecting spirometer, related to the cumulative volume of total expired gas; and the fall in end-tidal nitrogen concentration, also related to cumulative expired gas volume. The data are summarized in tables 5 through 7.

It may be seen in table 5 that, if the accumulation of spirometer nitrogen is considered by increments corresponding to equivalent incremental volumes of expired total gas, no significant differences can be found between the eupneic oxygen breathing at ambient pressure values and the IPPB with and without isoproterenol values. It will be noted that interval values be-

*Statistical analysis of all parameters dealt with in tables 5, 6, and 7 showed no significant differences between the first group of patients (*Nos. 1–6*) and the later group (*Nos. 7–12*).

TABLE 5

MEAN VOLUMES OF NITROGEN ACCUMULATED IN SPIROMETER AS VENTILATION WITH OXYGEN PROGRESSES

	ΔV_E	0–10	10–20	20–30	30–40	40–50	0–50	50–100	Total
Eupneic ambient pressure breathing	ΔV_{N_2}	1.33	0.81	0.49	0.33	0.24	3.20	0.46	3.56* 4.17†
Intermittent positive pressure breathing	ΔV_{N_2}	1.34	0.81	0.51	0.32	0.24	3.22	0.57	4.08†
Intermittent positive pressure breathing and isoproterenol	ΔV_{N_2}	1.30	0.88	0.51	0.34	0.24	3.27	0.43	3.38* 4.14†

Figures in first seven columns refer to liters of N_2, expressed as increments (ΔV_{N_2}), accumulated during indicated increments of ventilation with O_2 (ΔV_E). These N_2 values are based on analysis with N_2 meter and are corrected to STPD, after correction for inspired N_2 and for diffusion of N_2 from tissues.

Figures in last column refer to total volume of N_2, determined by Van Slyke analysis and corrected as above, accumulated during entire washout procedure.

* Group A, patients *Nos. 1–6*, in whom total V_E was approximately 100 L.

† Group B, patients *Nos. 7–12*, in whom total V_E exceeded 200 L.

Analysis of variance shows no significant differences in N_2 volumes for a given ventilation increment as a result of intermittent positive pressure breathing with or without isoproterenol.

TABLE 6

MEAN F_{AN_2} PRODUCED BY CUMULATIVE VENTILATION VOLUMES

	V_E	10	25	50	75	100	125	150	175	200	225	250
Eupneic ambient pressure breathing	F_{AN_2}	22.8	10.8	4.8	2.8	1.74	1.37	1.02	0.87	0.72	0.65	0.56
Intermittent positive pressure breathing	F_{AN_2}	24.2	12.8	5.6	3.1	1.94	1.35	0.95	0.72	0.54	0.53	0.45
Intermittent positive pressure breathing and isoproterenol	F_{AN_2}	24.1	12.1	5.1	2.6	1.49	1.08	0.74	0.57	0.45	0.37	0.21
P values												
Eupneic ambient pressure breathing vs. intermittent positive pressure breathing	*		*	*	*	*	0.30	0.31	0.27	0.06	0.06	†
Eupneic ambient pressure breathing vs. intermittent positive pressure breathing and isoproterenol	*		*	*	*	*	0.03	0.01	0.007	0.009	0.01	†
Intermittent positive pressure breathing vs. intermittent positive pressure breathing and isoproterenol	*		*	*	*	*	0.17	0.12	0.18	0.16	0.03	†

F_{AN_2} = Alveolar (end-tidal) N_2 concentration, expressed as per cent, corrected for N_2 in inspired O_2, but not corrected for diffusion of N_2 from blood.

V_E = ventilatory volume, or liters of expired gas.

P = probability that observed difference between the means could have occurred by chance. "P" equal to or smaller than 0.05 is considered significant.

* At these levels of ventilation no significant differences were detected by analysis of variance.

† Number of observations at this volume of ventilation were too few to permit statistical evaluation.

yond 100 liters of total expired gas are not given for the nitrogen meter method of analysis, the reason being that inaccuracy of the meter is greatly magnified beyond this point. (The error amounts to 0.08 liters of nitrogen at the 50-liter level, 0.16 liters of nitrogen at the 100-liter level, and 0.40 liters of nitrogen at the 250-liter level.) The values for total nitrogen displaced during the entire procedure by equivalent volumes of oxygen, based on actual Van Slyke analysis, also failed to show significant differences.

From this it may be suspected that, if hyperventilation (with or without isoproterenol) displaces more nitrogen for equivalent volumes of oxygen breathed, it will be demonstrated at ventilation volumes beyond 100 liters but before the total ventilation reaches the neighborhood

TABLE 7

MEAN VENTILATION VOLUMES REQUIRED TO LOWER F_{AN_2} TO 10, 1, AND 0.5 PER CENT

A.

	Initial F_{AN_2} to 10 per cent	Initial F_{AN_2} to 1 per cent	Initial F_{AN_2} to 0.5 per cent
Eupneic ambient pressure breathing	27.0	137.8	229.0
Intermittent positive pressure breathing	31.4	144.0	203.0
Intermittent positive pressure breathing and isoproterenol	29.6	121.5	181.0
P (variance among 3 trials)	>0.10	>0.10	0.05
P (for paired trials):			
Eupneic ambient pressure breathing vs. intermittent positive pressure breathing	*	*	0.10
Eupneic ambient pressure breathing vs. intermittent positive pressure breathing and isoproterenol	*	*	0.02
Intermittent positive pressure breathing vs. intermittent positive pressure breathing and isoproterenol	*	*	0.12

B.

	Initial F_{AN_2} to 10 per cent	10 per cent to 1 per cent	1 per cent to 0.5 per cent
Eupneic ambient pressure breathing	27.0	111.0	84.5
Intermittent positive pressure breathing	31.4	114.2	60.6
Intermittent positive pressure breathing and isoproterenol	29.6	91.2	48.6
P (variance among 3 trials)	>0.15	<0.05	<0.02
P (for paired trials):			
Eupneic ambient pressure breathing vs. intermittent positive pressure breathing	*	>0.05	0.01
Eupneic ambient pressure breathing vs. intermittent positive pressure breathing and isoproterenol	*	0.05	0.02
Intermittent positive pressure breathing vs. intermittent positive pressure breathing and isoproterenol	*	0.05	0.16

* No significant difference between the pairs (P = 0.05 considered level of significance).

of 250 liters, which was generally the termination point for the last 6 patients.

The nitrogen accumulation curve during eupneic oxygen breathing at ambient pressure for the group as a whole is illustrated in figure 3. The inflection point at 50 liters of ventilation corresponds to the displacement of 88 per cent of the total nitrogen recovered from the first 6 patients (in whom total washout volume averaged 97 liters), and to 78 per cent of the total nitrogen displaced in the last 6 patients (in whom total washout volume averaged 230 liters). It would appear to represent a dividing point between accumulation of more readily and less readily displaced nitrogen. In similar plots for individual patients, two or three such sharply breaking straight-line segments are usually seen. It is estimated that the mean nitrogen volume collected at this 50-liter inflection point represents 82.8 per cent of the mean functional residual capacity for the group APB trial.

Tables 6 and 7 contain data on the mean washout curves during APB, IPPB, and IPPB with isoproterenol. They relate the fall in end-tidal ("alveolar") nitrogen concentration to ventilation volumes, considered from two aspects. The nitrogen concentrations achieved at equivalent ventilation volumes are shown in table 6,

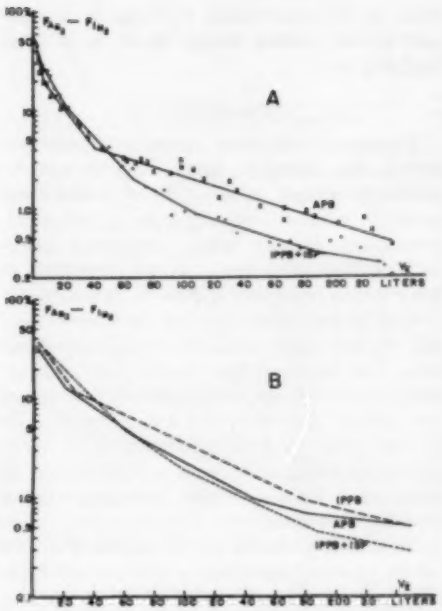

FIG. 4. (*A*) Nitrogen washout curves for patient *No. 7*. Note rather marked effect of intermittent positive pressure breathing with isoproterenol and the scattering of points through which the curves are drawn. (*B*) Nitrogen washout curves for patient *No. 11*.

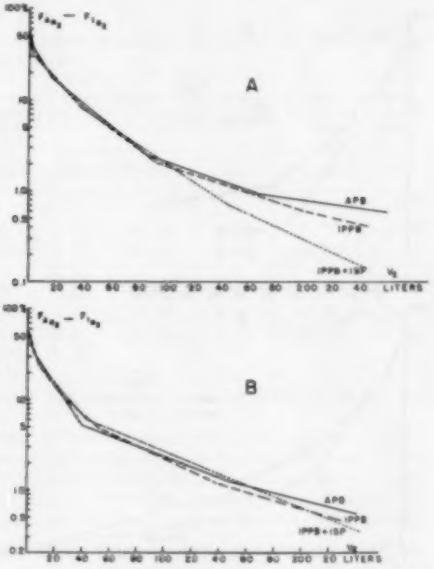

FIG. 5. (*A*) Nitrogen washout curve for patient *No. 8*. (*B*) Nitrogen washout curve for patient *No. 9*.

while the amount of ventilation required to produce equivalent reductions in nitrogen concentration may be noted in table 7. As is indicated in table 6, significant differences between the APB and IPPB with isoproterenol curves occur at and beyond the 125-liter ventilation point, while IPPB alone has a lesser effect. These trends may also be seen in table 7.[7] Recalling that approximately 80 to 90 per cent of the total nitrogen displaced is collected at 50 liters, it is apparent that the improvement in nitrogen clearance involves the most poorly ventilated spaces. A significant effect on the nitrogen clearance from the better ventilated spaces was not obvious, but may have been masked by a greater contribution from the less well-ventilated spaces during the earlier phases of the washout procedure.

In the group as a whole the improvement in nitrogen clearance is not striking (figure 2). Individual cases showed variations (figures 4, 5). By contrast, 2 patients with asthma showed marked responses to IPPB with isoproterenol (figure 6).

Aminophylline had no significant effect on tidal volumes or on nitrogen clearance, leading to the conclusion that alveolar ventilation relationships were not altered by this drug. Since no significant differences were found, detailed data for the aminophylline experiments are not shown. Indeed, so similar were the findings during oxygen breathing before and after intravenous aminophylline that the results were all pooled and used for comparison with the mean eupneic oxygen breathing at ambient pressure trials which preceded the hyperventilation trials in order to evaluate the reproducibility of the

[7] Correction of data in table 7 for functional residual capacity (FRC) variation by dividing the expired volumes by the functional residual capacity determined for each procedure (which yields a figure for V_B per liter of FRC) does not add to their significance. Probably this is due in part to the relatively small variation in FRC from procedure to procedure and, in part, to the fact that such correction implies that nitrogen clearance from the whole FRC is involved in the later stages of the washouts. Actually, in these later phases, only certain compartments or areas of the lung, which are fractions of the whole FRC, may contain nitrogen still to be cleared.

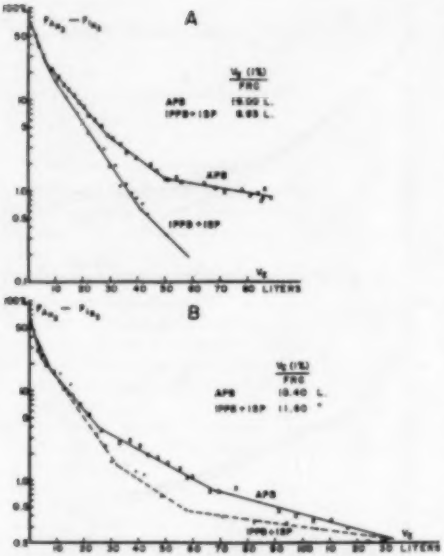

FIG. 6. (*A*) Nitrogen washout curves during oxygen breathing at ambient pressure and intermittent positive pressure breathing with isoproterenol for a twenty-four-year-old patient with asthma in apparent clinical remission. Intermittent positive pressure breathing with isoproterenol has produced a marked improvement in alveolar ventilation, indicating that the patient was not in complete remission. (*B*) Nitrogen washout curves for a thirty-year-old patient with asthma in partial remission. The effect of intermittent positive pressure breathing with isoproterenol is less marked than in the patient mentioned in (*A*).

APB results when done on different days. The degree of reproducibility found was good.

A comparison of the total duration of oxygen breathing for each procedure, and the amount of nitrogen washed out thereby, is given in table 8. It is to be noted that, while the differences are variable from patient to patient, the data suggest that more nitrogen was cleared during the longer APB trials than during the shorter IPPB trials, despite equivalence of volumes of oxygen breathed. This suggests that certain very poorly ventilated spaces may exchange their gas with the conducting airways by the slow process of diffusion.

The end-tidal and arterial carbon dioxide concentrations and arterial pH measurements determined in the first 6 patients during APB and IPPB with isoproterenol trials are summarized in table 4 and indicate the effectiveness of this form of hyperventilation in reducing alveolar and arterial carbon dioxide levels in a short period of time.

DISCUSSION

Experience with some young asthmatics has shown that nitrogen dilution curves can be strikingly altered as a result of intermittent positive pressure breathing plus isoproterenol, presumably because poorly ventilated spaces were enabled to receive a greater proportion of oxygen than previously (figure 6). It is a matter of considerable interest to find out whether similar changes might occur in the emphysematous lung. The results of the present study indicate that, in general, the ventilation relationships of the various alveolar spaces are not profoundly altered either by hyperventilation or by bronchodilator drugs, or by both in combination. In some cases no appreciable alteration whatsoever has been found.

Such findings should not be surprising in view of the profound distortion of alveolar and bronchiolar anatomy and mechanics in emphysema. That there is any improvement at all in ventilation of some of these spaces is of great interest. Obviously, the degree of uneven alveolar ventilation which is found in this disease is not closely related to regional or general bronchospasm. This may exist, but to a relatively small extent. The beneficial effect of isoproterenol may have been due as much to a shrinking of bronchiolar mucosa as to actual relaxation of bronchospasm. This is also suggested by the absence of obvious effect from intravenous aminophylline. That there is no delayed effect from isoproterenol which might not be apparent *during* an IPPB with isoproterenol procedure is indicated by the writers' experience in a few cases wherein the patients were subjected first to APB, then to IPPD with isoproterenol, and twenty to thirty minutes later had a repeat APB washout. No additional change was found in the third washout.

The rather striking improvement in nitrogen clearance in patient *No. 7* (figure 4) is difficult to explain, especially since an earlier study done on him had not shown so notable a result. This man subsequently died. Postmortem examination of his lungs (fixed in an inflated position with formalin fumes) revealed extensive generalized emphysema with alveolar wall disruption.

TABLE 8

TOTAL N_2 CLEARED BY EQUIVALENT TOTAL VENTILATION VOLUMES RELATED TO DURATION OF O_2 BREATHING

Patient Number	Eupneic Ambient Pressure Breathing		Intermittent Positive Pressure Breathing		Intermittent Positive Pressure Breathing and Isoproterenol		Eupneic Ambient Pressure Breathing before Aminophylline		Eupneic Ambient Pressure Breathing after Aminophylline	
	Duration	V_{N_2}	Duration	V_{N_2}	Duration	V_{N_2}	Duration	V_{N_2}	Duration	V_{N_2}
	min.	*L.*	*min.*	*L.*	*min.*	*L.*	*min.*	*L.*	*min.*	*L.*
7	30.78	3.79	—	—	6.32	3.90	29.65	3.51	26.77	3.55
8	21.52	5.01	14.12	4.73	10.42	4.76	24.58	4.42	20.50	4.62
9	20.47	4.58	15.99	4.36	12.15	4.43				
10	19.37	4.40	15.50	4.38	12.30	4.42	21.50	4.86	21.10	4.64
11	20.46	4.65	12.52	4.22	10.28	4.54	18.54	4.56	16.57	4.49
12	19.42	3.80	15.86	3.81	7.58	3.75	21.72	3.52	18.63	3.39
12	23.64	3.68	15.98	3.48	7.55	3.33				

V_{N_2} = volume of N_2 corrected to STPD after correction for inspired N_2 and for diffusion of N_2 from tissues. Based on Van Slyke analysis.

The eupneic ambient pressure breathing, intermittent positive pressure breathing, and intermittent positive pressure breathing and isoproterenol values are to be compared to each other for each patient; the eupneic ambient pressure breathing trials before and after aminophylline trials were done on a different day and are to be compared with each other for each patient. In all comparable trials for each patient, the same total ventilation volume was achieved, and in this group of patients this approximated 250 liters of O_2 breathing (*see* table 4).

Previous investigations on nitrogen clearance from emphysematous lungs have revealed the great delay which characterizes this disease (14–16, 21), and comparable findings have been reported with helium washout methods (22). Robertson, Siri, and Jones (23) and Fowler, Cornish, and Kety (14) have described methods of analysis of the washout curves which yield quantitative data on the size and rate of ventilation of well ventilated and poorly ventilated spaces. The initial steep parts of the curve represent clearance from well ventilated spaces while the latter slowly falling segments represent the contribution from the poorly ventilated compartments. Modifications of this approach are used by Blair and Hickam (22) and by Lundin and his associates (15, 32). These various writers have plotted fall in nitrogen or helium against time or number of breaths. Georg (16) points out that variations in rate and depth of ventilation will affect the time course of alveolar nitrogen dilution and plots nitrogen washout against expired volume, a procedure independently adopted by the present writers (18). The size of the poorly ventilated compartment in emphysematous lungs has been estimated to average from 53 to 78 per cent of the functional residual capacity (14, 15, 22, 24).

The effect of hyperventilation on pulmonary nitrogen clearance from normal lungs has interested several investigators (23, 25, 26). None have found that the clearance was consistently made more efficient by increasing tidal volume. Robertson, Siri, and Jones (23) concluded that hyperventilation improves ventilation in the better ventilated spaces, but does not affect the "deeper" or less well ventilated spaces, present even in normal lungs.

The effect of hyperventilation on clearance of helium from emphysematous lungs was reported by Lusk and associates (27), who found that unequal gas mixing persisted during exercise but did not become worse. More recently, Torres, Emerson, and Lyons (28) reported on the effect of IPPB on nitrogen clearance in ten patients with emphysema. A significant improvement in alveolar ventilation was found, manifested as a decrease in the number and size of poorly ventilated compartments and an increase in rate of ventilation of these spaces, as derived from the method of analysis of Fowler, Cornish, and Kety (14).

The data in the present paper have not been subjected to Fowler's method of analysis, chiefly because it depends rigorously on the slopes of the various lines drawn through the observed points. In actual practice, a large scatter of points is observed, especially during eupneic oxygen breathing at ambient pressure trials, and the scatter is magnified in the later phase of the

washout curve due to the use of a semilogarithmic scale for alveolar nitrogen concentration (figure 4). The drawing of a line through these points involves a subjective element. The scatter is attributed to variations in tidal volume from breath to breath. The theoretical basis for the mathematical analysis of the washout curves assumes constancy of tidal volume, lung volume, and dead space. Lack of reproducibility and disagreement as to procedure in the application of the analytic method of Fowler and his associates have been reported (15, 25). It has seemed to be more practical to present the washout curves in the form of decline of end-tidal nitrogen plotted against expired volume, with due acknowledgment that the curves as drawn represent estimates of best fit among the observed scattered points. Furthermore, a strong possibility exists that series ventilation as well as parallel ventilation occurs in the emphysematous lung; that is, the breakdown of alveolar walls must permit diffusion of gas from well ventilated alveoli to adjacent poorly ventilated alveoli, and vice versa. The mathematical handling of nitrogen clearance from such a system is different from that in a system of spaces ventilated in parallel (20, 23).

Comparing the washout curves of the present subjects during APB and IPPB with and without isoproterenol, it is noteworthy that deviations occurred in the latter part of the procedure, when the poorly ventilated spaces were being uncovered. The failure of change to be apparent in the early part of the curve was unexpected, since presumably this segment represented the well ventilated areas.

The fall in arterial carbon dioxide and rise in arterial oxygen saturation when emphysematous patients are hyperventilated by IPPB with air (6) can occur only because of improved ventilation in perfused alveoli. These presumably are the more intact alveoli and reside in the better ventilated compartments of the lung.

If such improvement in arterial oxygen and carbon dioxide indicates greater efficiency of ventilation in these more intact alveoli, why, then, do we not see an alteration in the early port of the nitrogen washout curve as a result of hyperventilation? Possibly the reason is that the better ventilated spaces are replenished with nitrogen from the less well ventilated spaces as rapidly as they are cleared, a concept previously stated by Roos, Dahlstrom, and Murphy (21). Such a situation would also account for the fact that later in the course of the hyperventilation lower nitrogen levels would finally become apparent. However, such a shift of nitrogen from poorly ventilated areas to better ventilated areas, masking the actual improved clearance in these latter alveoli, should be revealed as a greater accumulation of nitrogen in the collecting spirometer during the earlier phase of the IPPB procedure. This was not noted (table 5).

Another possibility is that the relative distribution of inspired oxygen to the various alveolar spaces and the relative turnover in these spaces cannot be rapidly altered by inhaled bronchodilator drugs as they can be in early asthma, owing to the anatomic changes; yet later in the washouts the inhaled isoproterenol may produce enough increase in bronchiolar caliber to facilitate better gas exchange than was possible when the IPPB with isoproterenol procedure began.

Still another possibility is that the increase in anatomic dead space which accompanies increased tidal volume may nullify or reduce the beneficial effect. However, the enlargement of dead space as tidal volume increases by 1 to 2 liters has been estimated to be in the neighborhood of 50 to 100 ml. (29, 30) or less (31), which should still leave a large volume of oxygen available for penetration into those alveolar areas which are more accessible by virtue of their geometric and mechanical properties.

Whatever may be the actual mechanism of the observed changes, it would appear that the clinical use of IPPB with isoproterenol to promote more efficient ventilation of alveoli in the emphysematous patient has some justification from the findings herein described, namely, the greater clearance of nitrogen from the more poorly ventilated spaces, an effect which is definite if not marked. The possibility of achieving rapid lowering of arterial carbon dioxide levels, an effect which probably relates to more efficient ventilation of the more intact alveoli, is of greater immediate interest to the clinician than the demonstration that poorly ventilated spaces can be flushed of their nitrogen, unless subsequent work shows that these spaces are sites of sizeable physiologic shunts of blood from the venous to the arterial side of the circulation. These possible advantages from IPPB with isoproterenol in improved alveolar gas exchange

are not considered in the light of the work involved for the patient (33).

Summary

A study was made of the effects of hyperventilation and of bronchodilator drugs on alveolar ventilation in 12 patients with emphysema. Hyperventilation was produced by intermittent positive pressure breathing (IPPB), with and without aerosolized isoproterenol, and the course of alveolar nitrogen displacement by inspired oxygen was followed in relation to the volume of oxygen breathed. Comparison was also made of pulmonary nitrogen clearance before and after intravenously administered aminophylline.

Alveolar ventilation, as judged by nitrogen clearance, was less improved by hyperventilation alone (IPPB without isoproterenol) than by hyperventilation accompanied by aerosolized bronchodilator drug (IPPB with isoproterenol). Nitrogen clearance was not improved by intravenously administered aminophylline. These results suggest that the beneficial action of inhaled isoproterenol may be due more to topical shrinking of bronchiolar mucosa than to relaxation of bronchospasm.

The improvement in alveolar ventilation of the lung as a whole resulting from IPPB with isoproterenol is definite but not marked, a finding not surprising when the distorted bronchiolar and alveolar anatomy in emphysema is considered. Nevertheless, there appears to be justification for using such treatment in this disease.

Sumario

Efecto de la Respiración a Presión Positiva Intermitente y de los Broncodilatadores sobre el Despejo del Nitrógeno Alveolar en los Enfermos de Enfisema Pulmonar Oclusivo Crónico

Este estudio comprendió los efectos de la hiperventilación y de los broncodilatadores sobre la ventilación pulmonar en 12 enfisematosos. Se produjo la hiperventilación por la respiración a presión positiva intermitente (RPPI) con o sin isoproterrenol aerosolizado y se observó la tendencia del desplazamiento del nitrógeno alveolar por el oxígeno aspirado en relación con el volumen del oxígeno respirado. También se hizo una comparación del despejo del nitrógeno pulmonar antes y después de administrar aminofilina intravenosamente.

Juzgada por el despejo del nitrógeno, la ventilación alveolar mejoró menos con la hiperventilación sola (RPPI sin isoproterrenol) que con la hiperventilación acompañada de un broncodilatador aerosolizado (RPPI más isoproterrenol). No mejoró la eliminación del nitrógeno con la administración intravenosa de aminofilina. Sugieren estos resultados que el efecto beneficioso del isoproterrenol inhalado puede deberse más a la contracción tópica de la mucosa bronquiolar que a la dilatación del broncospasmo.

El mejoramiento de la ventilación alveolar del pulmón en conjunto debido a la RPPI más isoproterrenol es bien definido pero no destacado, hallazgo este que no debe sorprender cuando se considera la deformada anatomía bronquiolar y alveolar en el enfisema. No obstante, parece que tiene justificación el uso de este tratamiento en dicha dolencia.

Resume

Effet de la respiration intermittente en pression positive et des médicaments bronchodilatateurs sur la "clearance" alvéolaire de l'azote, chez des malades ayant un emphysème pulmonaire chronique obstructif

Les auteurs ont fait une étude sur les effets de l'hyperventilation et des médicaments bronchodilatateurs sur la ventilation alvéolaire, chez 12 malades ayant de l'emphysème. On produisit l'hyperventilation par la respiration intermittente en pression positive (IPPB), avec et sans aérosol d'isoproterenol, et on suivit le cours du déplacement de l'azote alvéolaire par l'oxygène inspiré, par rapport au volume d'oxygène respiré. On fit aussi une comparaison de la clearance de l'azote pulmonaire avant et après une injection intraveineuse d'aminophylline.

La ventilation alvéolaire, jugée d'après la clearance de l'azote, fut moins augmentée par l'hyperventilation seule (IPPB sans isoproterenol) que par l'hyperventilation associée à un aérosol de médicament bronchodilatateur (IPPB plus isoproterenol). La clearance de l'azote ne fut pas améliorée par l'administration intraveineuse d'aminophylline. Ces résultats font penser que l'action bénéfique de l'isoproterenol inspiré peut être due plus au rétrécissement local de la muqueuse bronchique qu'à la levée du spasme bronchique.

Dans la respiration alvéolaire de la totalité du poumon, résultant de l'IPPB plus isoproterenol, l'amélioration est certaine mais non marquée, donnée qui ne surprend pas lorsqu'on considère les déformations des bronchioles et des alvéoles dans l'emphysème. Néammoins, il semble qu'il est justifiable d'employer un tel traitement dans cette maladie.

Acknowledgments

The writers wish to express their appreciation to Dr. J. W. McKelligott for his statistical analysis of the data. The technical assistance of Miriam Runyon and David Barton and the help of Clara White in preparation of the manuscript are gratefully acknowledged. Dr. David Salkin originally suggested this project and has supported it throughout.

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ANTIBODIES TO HUMAN LUNG IN PATIENTS WITH OBSTRUCTIVE EMPHYSEMA AND PULMONARY TUBERCULOSIS[1,2]

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(Received for publication August 1, 1960)

Introduction

The remarkable work of Witebsky and associates (1) and Roitt and Doniach (2) demonstrating antibodies to thyroid extracts in experimental and clinical chronic thyroiditis has stimulated much work in the area of immune reactions with human organ or tissue extracts in various disease states. Similar antibodies have also been demonstrated in disseminated lupus erythematosus, glomerulonephritis, and various hematologic disorders. Much of this work has been well summarized recently (3). However, studies of immunologic phenomena in chronic pulmonary diseases have been almost completely limited to serologic tests for specific infectious agents and to skin tests for cellular hyperreactivity to these agents.

Since it has been well demonstrated (4, 5) that living or dead tubercle bacilli may markedly stimulate antibody response to weak antigens, it seemed reasonable that tubercle bacilli in infected lung tissue might serve as an "endogenous Freund adjuvant" enhancing antibody response to lung tissue altered by infection. Idiopathic obstructive emphysema was also studied because of some clinical similarities to emphysema, which occasionally follows extrinsic asthma, in which antigen-antibody reactions are thought to be of definite importance.

Method

The antigens were prepared from tissues obtained after death from patients without pulmonary disease. Peripheral lung tissue was washed in distilled water to remove blood, then homogenized as a 10 per cent suspension with cold normal saline. The homogenates were shaken overnight at 4°C. The supernatant was centrifuged at 3,000 r.p.m. to remove gross particles, and was frozen prior to use. One milliliter of this cloudy saline extract was added to 4 ml. of phosphate buffer at pH 7.4, and 0.814 μ latex particles were added to give a suspension of standard optical density. After incubation for one hour at 37°C., 0.1 ml. of the mixture was added to serial dilutions of test serum. The sera were then incubated at 37°C. for one hour and left overnight at room temperature. The tubes were centrifuged at 2,400 r.p.m. for three minutes, and read after slight shaking with the aid of a plane mirror. Reactions were considered positive when definite agglutination was seen in a 1:10 dilution of serum.

Results

In tests conducted with extracts made from two different lungs, a positive reaction was seen in only 2 of 60 control subjects without significant pulmonary disease. Thirty-three of the control patients were hospitalized and the remainder were apparently healthy subjects. The diseases found in patients in the control group are listed in table 1. Six of the control patients had fever. One of the 2 control subjects who gave a positive reaction had diabetes with Kimmelsteil-Wilson syndrome and was in terminal uremia. One had postnecrotic cirrhosis and subacute bacterial endocarditis. In contrast to the control group, 50 of 64 patients with tuberculosis who had roentgenographic evidence of cavities at the time of study showed definite positive reactions. Seven of 16 patients with active tuberculosis, but without definite roentgenographic evidence of cavity at the time of study, showed positive reactions. Positive reactions were found in 15 of 32 patients with obstructive emphysema of unknown cause. All three groups showed a significantly higher percentage of positive reactions than did the control group. The incidence of positive reactions was also significantly higher in the group with cavitary tuberculosis than in the group with no cavities ($P < 0.01$). Titers in the various sera varied from 1:10–1:1,280, the majority between 1:40 and 1:80.

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[2] Presented in part before the Medical Session, as part of *Section 7B*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 18, 1960.

Specimens of serum from 7 patients with active rheumatoid arthritis were also studied. Six gave positive reactions, with titers as high as 1:1,280. The results in patients with tuberculosis, emphysema, and rheumatoid arthritis are presented in table 2.

Serum from one patient with cavitary coccidioidomycosis and serum from 8 patients with histoplasmosis, 4 of whom had cavities, were also studied. Only one of the 4 specimens from patients with cavitary histoplasmosis reacted with the lung antigen. None of the other sera gave a positive test.

An apparent prozone was noted in the serum reactions from approximately one-half of the patients with obstructive emphysema, but was found in only 7 patients with tuberculosis. The apparent prozone disappeared when emphysema serum was heated for fifteen minutes at 56°C. (table 3). However, no previously negative serum became positive on heating for fifteen minutes. It seems likely that this phenomenon is similar to that noted by Schubart in serum from patients with rheumatoid arthritis, and may be due to an inhibitor in serum complement (6).

Serum from 19 patients with tuberculosis was tested for antibodies using an antigen made from fetal lung. Ten gave positive tests; 9 gave negative tests. Of the serum from patients with cavitary tuberculosis tested with this antigen, 9 were positive and 7 negative. Seven of the 9 sera which did not react with fetal lung antigen had previously reacted with antigen made from adult lung. One of the serum specimens which had previously failed to react with adult lung reacted with fetal lung. It appears that lung tissue obtained from an adult may be a slightly better antigen than fetal lung.

To determine the species specificity of this reaction, 10 positive tuberculosis sera were tested, using an antigen made from pork lung. Only one of the 10 reacted with this antigen, and the titer was much lower than with human lung antigen. These findings suggest that some species specificity is present.

Six positive serum specimens from patients with tuberculosis were tested using a latex antigen prepared with 1 ml. of 1:10,000 Old Tuberculin instead of 1 ml. of human lung antigen. None of these specimens reacted with this antigen.

Absorption studies were then done to deter-

TABLE 1

DISEASES PRESENT IN PATIENTS IN THE CONTROL GROUP

Disease	Number of Patients
Cirrhosis	5
Chronic pyelonephritis	3
Diabetes	2
Septicemia	2
Myocardial infarction	2
Carcinoma of prostate	2
Congestive heart failure	2
Granulocytic leukemia, malignant hypertension, hepatitis, duodenal ulcer, Hodgkin's disease, subdiaphragmatic abscess, Addison's disease, streptococcal tonsillitis, polyarteritis nodosa, rheumatic heart disease, thrombophlebitis, cerebral hemorrhage, chronic glomerulonephritis, abscess of leg, and bursitis	1 each
	33

TABLE 2

RESULTS OF SEROLOGIC TESTS

	Total	Positive	Negative	P
Control patients	60	2	58	
Patients with:				
Cavitary tuberculosis	64	50	14	<0.001
Noncavitary tuberculosis	16	7	9	<0.005
Pulmonary emphysema	32	15	17	<0.005
Rheumatoid arthritis	7	6	1	<0.001

TABLE 3

EFFECT OF HEATING SERUM FROM 2 PATIENTS WITH EMPHYSEMA AT 56°C. FOR 15 MINUTES

	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Unheated	−	−	−	+	+	±	±
Heated	+	4+	2+	+	+	+	±
Unheated	−	−	−	2+	+	+	+
Heated	+	2+	2+	+	+	+	−

− = No reaction
± = Questionable reaction
+ = Definite macroscopic floculation
2+ = Macroscopic floculation with clumps but cloudy supernatant
4+ = Macroscopic floculation with clumps and clear supernatant

mine grossly the specificity of this reaction. Kidney, lung, and liver antigens from two different cadavers were used in addition to pooled normal human serum.

Eight strongly reactive serum specimens from patients with cavitary tuberculosis and 4 positive specimens from patients with obstructive emphysema were absorbed overnight in the cold with 1 mg. of lyophilized kidney and lung antigen prepared from the same cadaver. In each instance, the antibody was completely or almost completely removed by the kidney antigen. However, antibody was absorbed somewhat better by this lung antigen from serum specimens from tuberculous patients than from those with emphysema.

Serum from 5 patients with rheumatoid arthritis, 5 emphysematous patients, and 4 patients with cavitary tuberculosis was absorbed with 1 mg. of lyophilized pooled human serum, or 1 mg. of lyophilized lung, liver, or kidney extract prepared from another cadaver. In each instance, antibody was completely or almost completely absorbed by the lung extract from all serum from patients with cavitary tuberculosis or emphysema. In contrast to previous absorption studies, however, the kidney extract was ineffective in absorbing antibody from sera from emphysematous patients, and absorbed antibody only from serum from one patient with tuberculosis. Absorption with liver extract and serum produced little or no change in titer in these specimens.

In contrast, antibody was completely or almost completely removed by liver extract or serum in all serum from patients with rheumatoid arthritis. In no instance was antibody titer significantly reduced by lung or kidney extract. It is probable that the positive tests seen in rheumatoid arthritis are due to contamination of the lung antigen with serum protein.

TABLE 4

ABSORPTION STUDIES USING LIVER, KIDNEY, AND LUNG ANTIGENS AND POOLED SERUM

	Serum	Liver	Kidney	Lung	
				Whole	Supernate from Trypsin Digestant
Tuberculosis	0	±	+(0)	+	0
Emphysema	0	0	+(0)	+	+
Rheumatoid arthritis	+	+	0	0	0

0 = No absorption
± = Slight absorption
+ = Complete or almost complete absorption

Trypsin digestion of lung was done according to the method of Cole and associates (7) and Goodman and Baxter (8). A portion of the same lung was treated in a similar fashion without addition of trypsin and was used as a control. One milligram of lyophilized supernatant from the trypsin-treated or control lung was added to serum from patients with rheumatoid arthritis, tuberculosis, or emphysema. The sera were absorbed overnight in the cold.

In serum obtained from 4 patients with emphysema, antibody was completely or almost completely absorbed by the supernatant from tryptic digestion of lung. In no instance was antibody titer reduced in serum from 4 patients with cavitary tuberculosis or in serum from 2 patients with rheumatoid arthritis. Supernatant from control heated lung was ineffective in absorption of antibodies. These results are summarized in table 4.

The lyophilized supernatant from tryptic digestion of lung was partially amorphous on microscopic examination. What appeared to be small fibers could also be seen. No elastic fibers were seen in unstained preparations, in preparations stained with hematoxylin and eosin, or in preparations stained with orcein. This material reacted strongly with periodic acid–Schiff and alcian blue stains, indicating that neutral and acid polysaccharides were present. Material which stains with Sudan black, but not with Sudan red or oil red O, could be demonstrated in the supernatant from tryptic digestion, indicating the presence of phospholipid, glycolipid, or free fatty acid.

DISCUSSION

It can be seen from these results that pulmonary tuberculosis is associated with a high incidence of antibodies to human lung. These results are similar to those reported by Rheins and Burrell (9), who demonstrated antibodies to rabbit lung in rabbits with experimental pulmonary tuberculosis. It is likely that lung tissue becomes antigenic only when some component has been altered by infection or exposure to inhalants, either by combination with haptens from bacteria or inhaled hydrocarbons. It seems

possible that the antibodies demonstrated in serum from patients with tuberculosis represent a response to damaged tissue, perhaps a perfectly normal response which will be demonstrated with ever-increasing frequency as more diseases associated with tissue destruction are studied. However, these antibodies may, by combination with antigen in tissue, cause further damage to the lung.

The finding of antibodies to human lung in serum of patients with idiopathic emphysema places this condition in the group of diseases of unknown cause associated with antibodies to organs or tissues. A similar hypothesis was proposed by Crowle (10) on the basis of experimental work in guinea pigs. Because the antigen appears to be present in high concentration in some kidneys as well as in the lung, it is not unreasonable to speculate that it may be related to the basement-membrane antigen found by Krakower and Greenspon (11) in kidney, lung, and other vascular tissues. The ability of supernatant from tryptic digestion of lung to absorb these antibodies suggests that the antigen may contain polysaccharide or lipopolysaccharide, at least as a hapten. The strong reaction of the lyophilized supernatant with periodic acid–Schiff and alcian blue stains is supporting evidence that polysaccharide or lipopolysaccharide is present. It is possible that this material may be derived from reticulin, since Milazzo (12) has demonstrated that reticulin still maintains its ability to absorb antibodies after tryptic digestion. Further, reticulin is PAS positive and contains large amounts of lipid and polysaccharide surrounding collagen-like fibers. It is of particular interest in this regard that LeBlond and Bertalanffy (13) have found that there is abundant reticulin in rat lung and that reticulin membranes form the framework of alveolar walls.

The presence of antibodies in pulmonary emphysema is of considerable interest since this finding indicates new areas for work on a problem of ever-increasing importance in our aging population. Whether these antibodies are of importance in the pathogenesis of this disease, or whether they are merely indicators of removal of damaged tissue, is at present impossible to state. Exact identification of this antigen may, however, allow determination of the chemical structure in lung altered by this disease; information which is not available at present.

Summary

Antibodies reacting with extracts of human lung are present in the serum of many patients with pulmonary tuberculosis and idiopathic obstructive emphysema. The findings suggest that tubercle bacilli may serve as an "endogenous Freund adjuvant" stimulating antibody response to damaged tissue. Antibodies have also been found in the serum of 6 of 7 patients with rheumatoid arthritis.

It is concluded from absorption studies that these antibodies are directed against different components of the lung antigen. Antibodies in serum from patients with tuberculosis and emphysema are absorbed by lung and by some kidney antigens. They are not absorbed by serum or liver antigens. On the other hand, antibody in serum of patients with rheumatoid arthritis is absorbed by serum and liver, but not by lung or kidney.

Antibodies in serum from patients with tuberculosis and emphysema differ in that the antibody is absorbed in emphysema, but not in tuberculosis, by supernatant from tryptic digestion of the lung. Because of its resistance to tryptic digestion and strong reaction with stains used to demonstrate polysaccharides and lipids, it seems likely that the antigen in emphysema contains lipopolysaccharides as a hapten. The antigen may be derived from lung reticulin. It is possible that these antibodies may be of importance in the pathogenesis of pulmonary emphysema.

Sumario

Anticuerpos para el Pulmón Humano en los Enfermos de Enfisema Oclusivo y Tuberculosis Pulmonar

En el suero de muchos enfermos de tuberculosis pulmonar y de enfisema oclusivo idiopático se encuentran anticuerpos que reaccionan con extractos de pulmón humano. Sugieren estos hallazgos que los bacilos tuberculosos pueden servir como "coadyuvante endógeno de Freund," excitando la respuesta en anticuerpo al tejido lesionado. También se han encontrado anticuerpos en el suero de 6 de 7 enfermos de artritis reumatoidea.

De los estudios de absorción se deduce que estos anticuerpos visan a distintos componentes del antígeno pulmonar. Los anticuerpos en el suero, procedentes de los enfermos de tuberculosis y de enfisema, son absorbidos por los antígenos pulmonares y por algunos renales. No son absorbidos por los antígenos séricos o hepáticos. En cambio,

el anticuerpo contenido en el suero de los enfermos de artritis reumatoidea es absorbido por el suero y el hígado, pero no por el pulmón o el riñón.

Los anticuerpos en el suero de los enfermos de tuberculosis y de enfisema discrepan en que se absorben en el enfisema, pero no en la tuberculosis, por la fracción sobrenadante derivada de la digestión tríptica del pulmón. Debido a su resistencia a la digestión tríptica e intensa reacción a los colorantes usados para descubrir polisacáridos y lípidos, parece probable que el antígeno en el enfisema contenga lipopolisacáridos en forma de hapteno. Este puede derivarse de la reticulina pulmonar. Es posible que estos amticuerpos posean importancia en la patogenia del enfisema pulmonar.

Resume

Anticorps du poumon humain chez des malades ayant un emphysème obstructif et une tuberculose pulmonaire

Des anticorps réagissant avec des extraits de poumon humain se trouvent dans le sérum de beaucoup de malades ayant une tuberculose pulmonaire et un emphysème obstructif idiopathique. Ces données font penser que les bacilles tuberculeux peuvent servir de "facteur adjuvant endogène de Freund" stimulant la réponse en anticorps du tissu atteint. Des anticorps ont aussi été trouvés dans le sérum de 6 de 7 malades ayant une arthrite rhumatismale.

Les auteurs conclut des études d'absorption que ces anticorps sont dirigés contre les différents composés des antigènes pulmonaires. Les anticorps dans le sérum des malades avec tuberculose et emphysème furent absorbés par le poumon et par quelques antigènes rénaux. Ils ne furent pas absorbés par des antigènes sériques ou hépatiques. D'un autre coté, l'anticorps de sérum de malades ayant une arthrite rhumastismale est absorbé par le sérum et le foie, mais pas par le poumon ni le rein.

Les anticorps du sérum de malades ayant une tuberculose et de l'emphysèma diffèrent en ce que l'anticorps est absorbé dans l'emphysème, mais pas dans la tuberculose, par le liquide surnageant obtenu après la digestion du poumon par de la trypsine. A cause de sa résistance à la digestion par la trypsine et sa forte réction avec les colorantes employés pour mettre en évidence les polysaccharides et les lipides, il semble probable que l'antigène de l'emphysème contienne un lipopolysaccharide comme haptène. Il est peut-être un dérivé de la réticuline du poumon. Il est possible que ces anticorps soient importants dans la pathogènie de l'emphysème pulmonaire.

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COMPLICATIONS IN THE CHEMOTHERAPY OF TUBERCULOSIS[1]

A Review with Analysis of the Experience of 3,148 Patients

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(Received for publication March 23, 1960)

INTRODUCTION

The treatment of tuberculosis has been completely revolutionized in the past thirteen years, during which effective chemotherapy has been generally available. It cannot be denied, however, that valuable and potent as these antituberculous drugs are, they have their drawbacks. Toxic manifestations occur, sometimes with permanent and most unfortunate results, and allergic reactions to streptomycin and PAS are among the main problems of modern therapy.

The writers have sought to discover just how commonly these complications arise by analysis of experience in the treatment of more than 3,000 patients. Such an investigation seemed desirable for, extensive though the literature is on this subject, it is surprisingly unhelpful. Most reports refer to single-drug or high-dosage regimens or deal with small numbers of patients. In other reports, prominence is given to rare complications or to minor disturbances; and in some reports toxicity has been recorded only when it has resulted in permanent cessation of treatment and withdrawal of the patient from a therapeutic trial. Examination of a large series over a long time seemed especially desirable, for only in this way is it possible to confirm the impression or dispel the illusion that these complications are becoming more or less frequent.

PATIENT GROUP

All patients admitted between 1953 and 1959 to St. Wulstan's Hospital, Malvern, and Newtown Hospital, Worcester, who received antituberculous chemotherapy have been included. They numbered 3,148; all were adults; and the majority were suffering from pulmonary disease. In the hope of presenting an intelligible, coherent picture, consideration has been given only to reactions sufficiently severe to cause at least temporary interruption of treatment with streptomycin, PAS, or isoniazid; other less important antituberculous agents have been ignored, most of which are more toxic and are only used in the presence of bacterial resistance to isoniazid, streptomycin, or PAS.

[1] From Worcester Royal Infirmary and St. Wulstan's Hospital, Malvern, England.

DRUG REGIMENS AND DOSAGE

Streptomycin calcium chloride or sulfate was given in a dosage of 1 gm. intramuscularly either daily or twice weekly, but the calcium chloride salt was abandoned early in the series as being decidedly more painful. Para-aminosalicylic acid was administered orally as the sodium salt, 5 gm. usually twice but sometimes four times a day, either in solution or as a powder in rice paper cachets. An isoniazid dosage of 100 or 150 mg. twice daily was usually employed and rarely did the dosage exceed 5 mg. per kg. of body weight per day. When given together, the PAS and isoniazid were often dispensed in cachets containing a mixture of both substances in order to ensure a two-drug regimen.

The three drugs were given in varied combinations and were never administered singly. During the last three years, treatment has usually been started with 1 gm. of streptomycin daily, 5 gm. of PAS twice daily, and 100 mg. of isoniazid twice daily, the streptomycin being discontinued after six, twelve, or twenty-four weeks. The isoniazid and PAS were continued thereafter in cachet form for twelve months or longer. Prior to 1956, other regimens in common use were: 1 gm. of streptomycin daily with 5 gm. of PAS two, three, or four times daily; 1 gm. of streptomycin twice weekly with 5 gm. of PAS twice daily; and 5 gm. of PAS twice daily with 100 mg. of isoniazid twice daily.

PATIENTS AT RISK

The same regimen was not necessarily maintained during the treatment of each individual patient. Because of this variation, the incidence of complications has been estimated in relation to the number of patients at risk from each drug rather than in terms of specific regimens. The numbers at risk were: 2,660 to streptomycin; 2,248 to isoniazid; and 2,940 to PAS.

Reactions

In 379 cases (12 per cent), 384 complications were observed which led to an interruption of treatment. The various types of reactions are listed in table 1 and are classified as allergic or toxic. Not infrequently, patients experienced the same type of disturbance on two or more occasions, but only the original episodes have been counted.

Allergic Reactions

Allergic reactions form the majority of reactions listed and occurred in 310 patients for an incidence of 9.8 per cent. Of the 2,660 patients at risk to streptomycin, 155 (5.8 per cent) were affected as were 211 (7.2 per cent) of the 2,940 patients who received PAS. In 53 cases reactions developed to both drugs, and in an additional 54 a double allergy seemed probable, but was not proved. Isoniazid allergy was very rare and occurred in only 3 patients (0.1 per cent), in all of whom hypersensitivity to streptomycin and to PAS was also present. As far as the material would allow, the incidence of drug allergy among patients receiving different regimens has been analyzed, and no significant variation was found. The incidence with streptomycin was the same whether it was given daily or less often; PAS reactions were no more frequent with 20 gm. daily than with 10 gm. daily, nor was PAS more of an offender as a companion to streptomycin than as a companion to isoniazid.

TABLE 1

Complications of Chemotherapy in 379 Cases, Attributed to a Drug or Drugs

	Streptomycin	PAS	Isoniazid	Streptomycin and PAS	Streptomycin and PAS, and Isoniazid	Total
Allergic reactions						
Rash with fever	27	45	—	33	3	108
Rash alone	41	70	—	8	—	119
Fever alone	31	31	—	12	—	74
Hepatitis	—	6	—	—	—	6
Pulmonary infiltration with eosinophilia	—	3	—	—	—	3
Total	99	155	—	53	3	310
Toxic reactions						
Vestibular dysfunction	43	—	—	—	—	43
Deafness	7	—	—	—	—	7
Gastrointestinal disturbance	—	9	—	—	—	9
Thyroid dysfunction	—	2	—	—	—	2
Peripheral neuritis	—	—	7	—	—	7
Toxic encephalopathy	3	—	1	—	—	4
Blood dyscrasia	—	—	—	1	1	2
Total	53	11	8	1	1	74

By far the commonest manifestations of drug allergy were fever and skin eruptions. These were present together in 108 patients; fever without rash occurred in 74, and rash without fever in 119 patients.

The febrile reaction was sometimes heralded by small elevations of temperature for a day or two, but very often there was no such warning and the temperature chart, previously normal, showed a sudden rise to 103° or 104° F. (44° or 45° C.). If treatment were stopped at once the temperature would return rapidly to normal, although sometimes defervescence required two or three days.

The skin reaction was sometimes erythematous only, but usually was maculopapular or urticarial and closely resembled the eruption of measles or scarlet fever. The rash was usually irritating and ranged in extent and severity from a small area of scarcely visible papules on the trunk, face, or a limb to a generalized erythema, occasionally with petechial hemorrhages.

These two chief symptoms of drug allergy, fever and skin rash, were accompanied by other complaints. Pruritus of some degree was often experienced, more with PAS than with streptomycin. In general, nausea, vomiting, paresthesia, dizziness, headache, suffusion of the conjunctivae, albuminuria, and rigors are common, but rapidly disappear on withdrawal of the drug. A generalized lymphadenopathy with blood changes and sometimes splenic and liver enlargement may occur and give rise to a clinical picture superficially similar to infectious mononucleosis. Cannemeyer, Thompson, and Lichtenstein (1) described this type of severe hypersensitivity in 14 of 5,000 patients taking PAS, but only one instance was recognized in the present material.

Allergic reactions characteristically occurred between the second and the seventh weeks of

treatment and all but 29 in this series started during this time. However, in persons previously sensitized by exposure, such reactions may follow the initial dose within a few hours, presenting with fever and urticaria. Immediate reactions may also occur in patients previously sensitized to related compounds, such as acetylsalicylic acid, or with a history of photosensitivity, as in this illustrative case.

H.M., a female patient, aged thirty-six years, gave a history of dermatitis in 1946, following the use of penicillin cream, and of a photosensitivity reaction while on holiday in Switzerland in 1955. When she was found to be suffering from pulmonary tuberculosis in June, 1956, treatment was commenced with 2.5 gm. of PAS and 50 mg. of isoniazid. Four hours later the skin of her head and neck, forearms, and hands became markedly inflamed; within twelve hours her features were unrecognizable and there was gross thickening and blistering of the skin of the affected areas. During recovery it was found that exposure to sunlight caused a temporary relapse, and she was therefore moved to a darkened room. Repeated efforts were later made to desensitize her to PAS but without success.

Exposure to sunlight, menstrual periods, and nontuberculous infection may precipitate an allergic reaction at any stage of therapy. Exceptionally, a typical episode may commence late in the course of treatment. This happened in 2 patients, each in the twentieth week of therapy. One reaction was caused by streptomycin and one by PAS.

There was a particular group, however, in which allergy manifested itself later, between the seventh week and the sixth month, and in which the clinical pattern was slightly different. There were 15 patients in this group; all but 2 were more than fifty years of age and all but 3 were male. It seems likely, however, that age rather than sex was the determining factor, there being but few women of this age group in the series. Streptomycin and PAS were equally responsible. In these patients, who gave a history of previous skin trouble, the onset of the reaction was not dramatic and the first symptom was an irritable eczematous rash which could often be made tolerable by promezathine while continuing chemotherapy. In most cases, however, this palliation soon proved inadequate, and continued treatment brought increasing distress, a more intense exanthem, and eventually a true exfoliative dermatitis. Exfoliation may be widespread or even generalized, and it is often the skin of the head and neck which is most affected. Very severe degrees of hypersensitivity can be provoked. Permanent injury followed such a reaction in one patient who developed corneal ulceration, scleritis, and keratitis punctata with partial impairment of vision. In 5 cases, blepharitis was a prominent symptom.

Among the late-occurring rashes it is worth mentioning that of a patient in whom lichen planus developed after twenty-six weeks of streptomycin treatment and resolved dramatically after withdrawal of the drug. Another patient during the seventh month of treatment developed giant urticarial wheals which lasted a few hours after each streptomycin injection.

A rarer manifestation of hypersensitivity is the *transient pulmonary infiltration* which is occasionally seen during allergic reactions to PAS. Chest roentgenograms taken at the height of symptoms show pneumonic opacities around the tuberculous lesions which can easily be confused with deterioration of the tuberculous process. Within a few days of stopping treatment, however, complete spontaneous regression occurs and the rate of resolution is even more dramatic with corticosteroid therapy. The pathologic basis of these opacities is uncertain, but their frequent association with a high eosinophilia in the peripheral blood suggests that they are probably due to eosinophilic infiltration or edema. These "allergic" pneumonias were observed in 3 of the patients and in each the eosinophilic count was high. Few such cases have been reported; Holmboe (2) found only ten in the literature, all caused by PAS, and added one more. Jackson (3) reported perifocal reactions in three patients occurring at the time of generalized allergic manifestations, but in these there was no rise in the eosinophilic count. Two cases caused by isoniazid in the high dosage of 20 mg. per kg. of body weight per day have also been described by Perreau, Fresneau, Boumard, and Brizaut (4). Since roentgenograms are seldom taken at the height of such episodes, it is impossible to guess the true incidence, but it seems unlikely to be high. This assumption gains support from the writers' experience in the roentgenographic examination of 24 consecutively treated patients with fever due to PAS in whom no roentgenographic change was found.

Eosinophilia may occur with any allergic phenomenon and may reach remarkable levels, but it is an inconstant finding. A low-grade eosino-

philia is not an infrequent concomitant of streptomycin therapy, but serial counts during streptomycin or PAS therapy are of no prognostic value; nor is the presence of eosinophilia of any value in the diagnosis of allergic reactions. Routine blood investigations, on the whole, are rarely helpful. Leukocytosis and leukopenia may both occur, but most patients have perfectly normal counts.

It is generally accepted that *jaundice* is one of the more serious complications of PAS therapy. Originally, this was thought to be a toxic hepatitis, but it now seems that, in many if not all cases, it is another sign of drug allergy (5–7). This hypothesis is supported by its frequent association with a skin rash or fever, the common time of onset about the fifth week, and the fact that desensitization by either the slow or steroid technique can be achieved in most cases. The jaundice is of the typical "hepatocellular" type with enlargement of the liver, positive thymol turbidity and flocculation tests, reversal of the albumin-globulin ratio and only slight elevation of the serum alkaline phosphatase. Although one death from acute hepatic necrosis has been reported (8), complete recovery is the rule and all signs of liver damage have usually disappeared within a month.

In the present series, PAS hepatitis was observed in 6 patients (0.2 per cent) but in only 4 was desensitization attempted.

Proof of allergy cannot be reliably demonstrated except by a provocative test dose of the drug in question. To risk anaphylactic shock in this way is intrinsically undesirable and, indeed, following such a dose, one patient (Case H.F.) suffered a fatal myocardial infarction. In the writers' experience alternative techniques, such as patch and intradermal tests, have been completely unreliable.

H.F., fifty-three years of age, developed a maculopapular rash and a pyrexia of 102°F. (38.9°C.) after four and one-half weeks of treatment with 1 gm. of streptomycin twice weekly and 5 gm. of PAS twice daily. After an interval of fourteen days, he was given a test dose of 0.1 gm. of streptomycin by intramuscular injection and one hour later he was found in a collapsed state, sweating profusely, with a rapid thready pulse and a blood pressure of 80 systolic, 70 diastolic in mm. of mercury. An electrocardiogram taken six hours later was suggestive of a posterolateral myocardial infarction. Despite treatment, his condition gradually deteriorated, he lapsed into coma, and died on the following day. Postmortem examination revealed a hemorrhagic infarction involving the interventricular septum and the posterior wall of the left ventricle. There was also marked edema of the brain with some small hemorrhages in the pons.

The relationship of the myocardial infarction to the drug administration may have been coincidental, but it is recognized that such a test dose can cause a severe degree of shock, which in its turn could conceivably precipitate a myocardial infarction.

Recently, a more promising method for detection of drug allergy has been claimed by Hansen (9), who suggests the addition of a small amount of patient's serum to the solution for intradermal testing. Ley and associates (10) described a method of demonstrating specific antibodies to penicillin which was based on a chance finding of an agglutinin active against erythrocytes which had been stored in penicillin. It seems possible that some serologic test along these lines might make identification of drug allergy possible. Thus far, in a very few cases, the present writers have had no success with this method.

The provocative test used in this series was to give 0.1 gm., 1.0 gm., and 5.0 gm. of PAS by mouth on successive days, or 0.1 gm. followed by 1.0 gm. of streptomycin by intramuscular injection, the offending drug being identified by the recurrence of a mild reaction. When hypersensitivity to both agents seemed possible, test doses of both were usually given. In some cases, routine desensitization to both drugs was attempted, starting with PAS, without seeking to establish cross sensitivity.

As the proper treatment of the tuberculous disease depends on the early renewal and continuation of the chemotherapy, desensitization should start as soon as the fever and rash have settled and the cause is known. The writers have not encountered difficulties such as were reported by McLeod (11) in attempting desensitization after a much longer interval. As a rule, every effort should be made to re-establish tolerance to PAS because long-term outpatient therapy is so dependent on this drug. With streptomycin, circumstances may arise in which desensitization seems unnecessary or impracticable, particularly when the reaction occurs toward the end of the elective course. For this and other reasons, desensitization was not attempted in 39 of the 310 patients in whom hypersensitivity occurred. The attempt was unsuccessful in 6 of the remainder, and apparently successful

in 265 patients. The first attempt at desensitization was not invariably a success.

There is a choice of two methods whereby desensitization may be attempted: a gradual increase from minute to full doses over ten to fourteen days, or early resumption of chemotherapy accompanied by corticotropin or prednisolone. The first method, a modification of that described by Crofton and Foreman (12) was apparently successful in 233 patients, but failed in four. The initial dose was 0.1 gm. of PAS or 25 mg. of streptomycin, and the amount was increased gradually each day until 10 gm. of PAS or 1 gm. of streptomycin was tolerated. Usually, the course was uneventful, and no further trouble arose, but, in some cases, because of continued intolerance, very slow increases were required; and in 22 patients (9 per cent) allergic reactions recurred two or three weeks after the re-establishment of full dosage, necessitating a further course.

In this series only limited use has been made of corticosteroids and no attempt to judge the relative merits of the two methods would be justified. In 19 cases full dosage of the offending drug was successfully resumed in association with 30 to 40 mg. of prednisolone daily. The dosage was reduced to a suitable maintenance level after approximately seven days and was continued for four to six weeks, as allergic reactions to the drug tend to recur if prednisolone is stopped prematurely or abruptly. Corticotropin was also used in this way according to the method described by Houghton (13) who recommended a high-dosage scheme tapering off over six weeks, but the results obtained in 15 cases did not appear to justify the potential dangers and side effects. In 2 patients reactions recurred while they were receiving 80 units daily of corticotropin and reappeared in 2 others at the end of the steroid course. Recently Francis and Gupta (14) and Gupta and Palmer (15) have claimed successful desensitization in 16 cases with short, five-day courses of corticotropin accompanied by reinstitution of the antituberculous chemotherapy on the third day. The reported results with cortisone and its synthetic analogues have been less promising, but very large doses of prednisolone, up to 120 mg. daily, may be even more effective than corticotropin, which appears to vary greatly in antiallergic potency and perhaps is too dependent for its effect on the patient's own power to respond to its corticotropic stimulus. Certainly, it has been possible to continue the tuberculosis chemotherapy when accompanied by prednisolone in high dosage in one patient after corticotropin had failed to control the allergic symptoms.

Toxic Reactions

Streptomycin

Since the introduction of streptomycin, vestibular dysfunction and deafness have been recognized as its most serious drawbacks. The incidence of neurotoxicity is directly related to the size of the daily dose and the duration of therapy (16). There was hope at one time that the dihydro derivative would reduce this neurotoxicity, but by 1953 it was abundantly clear that, although vestibular dysfunction was less common than with streptomycin, there was an increased risk of permanent deafness from dihydrostreptomycin. For this reason, dihydrostreptomycin was not used in this series.

Vestibular dysfunction: Streptomycin was discontinued because of vestibular dysfunction in 43 (1.6 per cent) of the 2,660 patients at risk. It is necessary, in this connection, to distinguish between the regimens used, for this complication did not occur among the 696 patients treated with streptomycin administered less frequently than daily. With these cases excluded, the incidence of vestibular dysfunction for daily streptomycin was 2.2 per cent. This is a much lower figure than has been reported in other series. The difference may be partially explained by the fact that a considerable number of these patients received short courses of no more than 42 gm. A higher incidence is obtained if the last 514 consecutively treated patients on a planned regimen of daily streptomycin for at least three months are studied, for 31 of the 43 cases of vestibular dysfunction occurred in this group, giving a figure of 6.0 per cent. This incidence is to be compared with the reported rates of: 12 per cent of 107 patients reported by the Medical Research Council (17); 20 per cent of 60 patients by Lumsden and Powell (18); 24 per cent of 195 patients by Cohen, Johnson, Lichtenstein, and Lynch (19); 21 per cent of 149 patients by Mahady, Armstrong, Beck, Horton, and Lincoln (20); and 14 per cent of 222 patients by Penman, Dickson, and Miller (21). In the present series, however, tests of vestibu-

lar function were not carried out routinely in asymptomatic patients.

The incidence of vestibular dysfunction by sex and age showed a preponderance in the middle-aged and elderly men. Almost one-half of the cases occurred in men more than forty-five years of age. This cannot be claimed as evidence of increased susceptibility with aging because, in the absence of tests of labyrinthine function, slight degrees of damage may have been masked in younger patients by their greater ability to compensate for loss of vestibular function. The high incidence in the older age groups does, however, reflect the increased danger of significant disability in the elderly because of their decreased power of adaptation. The only other group to show an incidence above average was that of women less than 25 for reasons that are unclear.

The early signs of vestibular dysfunction are sometimes difficult to recognize in a patient confined to bed, and occasionally gross damage may occur before the patient complains or before the diagnosis is made. If a patient complains of giddiness on movement of the head or inability to read because of blurred vision, a full examination of vestibular function is mandatory. In the absence of any other reasonable explanation for sensations of vestibular dysfunction, streptomycin should be stopped at once. The presence of nausea, vomiting, lateral nystagmus, an unsteady widely based gait, and lack of stability on sudden turning of the head offer strong confirmatory evidence of vestibular involvement.

Vestibular dysfunction has been reported by Cawthorne and Ranger (22) after only 3 gm. of streptomycin in the presence of severe renal insufficiency and after 10 gm. or less in patients with normal renal function. In the present series, the dysfunction usually developed between the third and eighth weeks of therapy, but occasionally it appeared later. The average time of onset was 6.5 weeks after starting treatment, that is, after a total dose of 45 gm. of streptomycin. It is well recognized that this reaction is related to both the size of the daily dosage and the length of time during which it is administered.

Prompt recognition of the symptoms of vestibular dysfunction caused by streptomycin is almost always followed by a complete and reasonably rapid recovery in patients less than forty years of age. If the diagnosis is delayed, and in older people (particularly in those more than sixty), recovery is slower and often incomplete. Three or more weeks may pass before the actual giddiness subsides, and disability varying from slight instability in the dark to a severe ataxia may result. After withdrawal of streptomycin, however, the disorder does not become worse and, by using their vision to compensate for lack of balance, even the more severely afflicted can usually achieve some measure of mobility. Four patients in this series suffered serious and permanent damage.

The use of 150 mg. of pantothenic acid daily has been advocated, both as a possible means of preventing vestibular damage and in the treatment of the established disability, but there is at the present time no convincing proof of its efficacy in either respect. In 2 of the patients who were seriously and apparently permanently disabled, its administration was attended by dramatic improvement, but in others little or no benefit accrued. With the aid of this vitamin, Penman, Dickson, and Miller (21) were able to resume treatment after the abatement of symptoms in thirty of thirty-one patients, but their late results have been less satisfactory (23). Except in a few young women, the experience of the present writers with their method has been disappointing. Unfavorable results have also been reported by Raleigh (24) who compared streptomycin pantothenate with streptomycin sulfate in a double-blind controlled trial in 220 patients and found no difference between the two preparations.

Nerve deafness: The intramuscular administration of streptomycin daily caused impairment of hearing in 5 men and 2 women; all were more than forty years old and had normal renal function. The onset was often insidious and impossible to detect unless, as happened in 2 cases, vestibular dysfunction occurred at the same time. However, most writers agree that, on a constant daily dosage of streptomycin, ototoxicity is a later phenomenon than vestibular derangement (25). The earliest onset of deafness in the present series was after eight weeks of treatment (56 gm. of streptomycin). Loss of high frequency sounds was not usually noticed before the first ten to fourteen weeks of treatment, but in one case the first signs did not develop until the fifth month. It has been stated (26) that tinnitus often precedes loss of hearing, but this

was not the experience in the present series. In 2 cases tinnitus became a major complaint as the deafness slowly progressed, but it did not precede the hearing defect.

Progressive degeneration of auditory function may occur despite the withdrawal of the drug, and there is no recovery. Severe deafness resulted in 5 patients, and only 2 who experienced this toxicity escaped with reasonably satisfactory auditory function.

Para-Aminosalicylic Acid

Gastrointestinal manifestations: The common toxic manifestations of PAS are anorexia, nausea, vomiting, and diarrhea. These are generally ascribed to chemical irritation of the mucosa of the gastrointestinal tract, and seem to be directly related to the dosage employed. Under hospital conditions these symptoms are rarely troublesome enough to require interruption of treatment, and in only 9 cases (0.27 per cent) was it necessary to abandon the use of the drug altogether. Occasionally, the symptoms may occur as part of a frank allergic reaction, and in such cases it is possible to readminister the drug after carrying out a desensitization procedure.

After discharge from the hospital, gastrointestinal intolerance is both common and troublesome. Because of incomplete follow-up observations, the extent of this problem cannot be assessed from the present material. In a representative sample, roughly 10 per cent developed gastrointestinal symptoms during the later stages of prolonged administration of PAS. By manipulation of the dosage schedule, such as reducing it to a 10-gm. daily minimum, the institution of a weekly rest day, changing from one preparation to another, and firm encouragement, the majority could be persuaded to complete the prescribed course. In some patients this was not possible, however, and a few stopped taking the drug of their own volition, while protesting to the contrary, as established by negative chemical tests for PAS in the urine. Antacids and sedative mixtures were of little value in ameliorating this form of PAS toxicity, but it seems from published reports that chlorpromazine (27) and Antrenyl® (28) might be more helpful.

One factor in this higher PAS intolerance among outpatients may be the degeneration of PAS with age. Because of the rapid turnover, PAS prescribed in hospitals is usually used within a short time of manufacture. It has been observed, however, particularly in country districts where the demand is limited, that old stocks may be issued by the dispensing chemist. Mitchell, Schmidt, Capel, and Scheel (29) and Berte, Dewlett, and Schmidt (30) point out that PAS is a relatively unstable compound, susceptible to light, heat, and moisture, and that degradation products are produced which cause or enhance its irritant effect.

Goitrogenic action: The mechanism of the goitrogenic action of PAS depends on its inhibition of the organic binding of iodine in the synthesis of thyroid hormone (31), and the incidence of thyroid enlargement has a direct relationship to the dosage employed. With 20 gm. of PAS daily for five months or more, McGregor and Somner (32) reported enlargement of the thyroid, often with signs of myxedema, in 20 (23 per cent) of 83 tuberculous patients. In the present series in which 10 gm. daily was the standard dose for long-term treatment, only one case of goiter and one of myxedema were recorded, an incidence of 0.07 per cent. Thus, although minor degrees of enlargement may have been missed, dysfunction of the thyroid gland would seem to be a rare complication of PAS therapy except when large doses are given over a prolonged period.

Blood coagulation: A fall in the plasma prothrombin and proconvertin concentrations has been reported in patients receiving 12 gm. of PAS a day (33), but an extreme decrease, sufficient to incur a risk of hemorrhage, has been stated to be exceptional. On the other hand, Norviit and Carstensen (34) carried out 2,000 estimations of the prothrombin time without finding any deviation from normal in 400 patients receiving 0.2 gm. of PAS per kg. of body weight for periods up to a year. The same result was obtained from serial estimations in many of the patients in the present series, and major surgical operations were performed on 800 patients without any evidence of hemorrhage due to a coagulation defect.

Isoniazid

One of the outstanding properties of isonazid is its virtual lack of significant toxicity when used in a dosage of 2 to 5 mg. per kg. of body weight, which for the average subject is 200 to 300 mg. daily. Indeed, little is known of the in-

cidence of toxicity at this dosage level, for most reports in the literature describe complications at a dosage ranging between 6 and 14 mg. per kg. of body weight. Moreover, many of these patients were rendered more susceptible to drug toxicity because of impaired renal function (35), dietary deficiency, chronic alcoholism (36), epilepsy (37), and mental disease (38).

Peripheral neuritis: Peripheral neuritis, the most common complication of isoniazid therapy, appears to occur most readily with high dosage and particularly in those patients who acetylate isoniazid poorly (39). Biehl and Vilter (40) noted the similarity between the peripheral neuritis of isoniazid and of pyridoxine deficiency, and found in experimental animals a striking increase in the urinary excretion of the pyridoxine and pyridoxic acid, the increase being proportionate to the dose of isoniazid employed. There is some evidence that pyridoxine is not the only member of the vitamin B group which may become deficient during isoniazid therapy, for cases of typical pellagra have been described which have responded to nicotinamide (41, 42).

In the present series, peripheral neuritis was diagnosed seven times, or in 0.3 per cent of those treated with isoniazid. In only 3 patients had the standard dose of less than 5 mg. per kg. of body weight been employed. One of these patients was a chronic alcoholic with a mild preexisting neuritis which increased rapidly in extent and severity after the patient took 200 mg. of isoniazid daily for four days. For varied reasons, a higher dosage of 6 to 7 mg. of isoniazid per kg. was given to the other 4 patients without the simultaneous administration of pyridoxine, which is now known to prevent isoniazid neurotoxicity (43). It is worthy of mention that, in addition to these cases, acute polyneuritis has been observed in 3 patients treated with 1.2 gm. daily of the isoniazid analogues, glucuronolactone isonicotinyl hydrazone and *o*-hydroxy benzal isonicotinyl hydrazone, one of whom presented the full clinical picture of pellagra.

Tingling sensations and burning feet, associated with loss of vibration sense, were commonly the first signs of isoniazid neuritis. The symptoms appeared after at least six weeks of treatment, except in the case of the chronic alcoholic subject just reported. In 2 patients, intense burning pain was a characteristic and was limited to the lower legs and feet, which were hot, red, and swollen, although paresthesia and objective sensory changes were more widely distributed. Another patient had a mild optic neuritis, but fortunately this did not progress to optic atrophy as happened in 2 patients reported by Kass, Mandel, Cohen, and Dressler (44). As a general rule, complete recovery is the natural outcome on stopping treatment in most cases of isoniazid toxicity, even without the administration of vitamin supplements.

Toxic encephalopathy: A number of cases of toxic encephalopathy caused by isoniazid have been reported (42, 45–51). With the customary isoniazid dosage, however, this complication must be quite rare. Addington, Winfield, and Phillips (52), after a careful study of 52 patients with serial electroencephalograms and psychologic tests, were unable to demonstrate any definite neurotoxic effects of isoniazid, and concluded that, with therapeutic doses, it must be the exception for the psychotic manifestations to occur. In the present experience, there was only one patient, a 30-year-old female with acute schizophrenia, in whom isoniazid toxicity was a possible cause or precipitating factor. Withdrawal of the drug, however, had no effect on her mental condition, but eventually she made a good recovery after electro-convulsive therapy. There were 3 other instances of acute psychotic illness in patients who were treated with streptomycin and PAS without isoniazid, and it was thought that streptomycin might be responsible, for toxic dementia due to this drug has been described by Hare (51) and others. However, in these as in many published cases, a causal relationship was not established. It seems equally reasonable to assume that some persons with unstable personalities may break down mentally under the stress of long-term institutional treatment for tuberculosis.

Blood dyscrasias: Isolated cases of severe blood dyscrasia have been described as occurring because of isoniazid, PAS, or streptomycin. Review of the limited literature on these rare but often fatal complications suggests that streptomycin and isoniazid are perhaps more frequently involved than PAS, but any one of the three drugs has been reported to cause hemolytic or aplastic anemia, thrombocytopenic purpura, agranulocytosis, or pancytopenia. In the present series, one mild case of thrombocytopenia occurred as well as a case of agranulocytosis, which is described below in greater detail.

E.G., a 54-year-old man, was admitted to the hospital on March 26, 1956, with limited but active pulmonary tuberculosis and an eight-month history of epigastric pain thirty minutes after meals which was relieved by alkalis. Because of this history, a gastric diet was prescribed and PAS was not used during the first five weeks when he received 1 gm. of streptomycin and 200 mg. of isoniazid daily. Ten grams of PAS daily were then introduced into the regimen (May 2, 1956), isoniazid was continued, and the streptomycin injections changed from daily to twice weekly. On June 12, 1956, the patient complained of nausea, vomiting, and giddiness following a streptomycin injection, his temperature rose to 104°F., and all drugs were immediately stopped on the presumptive diagnosis of a hypersensitivity reaction. However, the fever and illness continued, a large ulcer developed on his palate, and a leukocyte count on June 15, 1956, revealed only 520 leukocytes per cu. mm. in the peripheral blood, all of which were lymphocytes. In the sternal marrow, there was complete absence of granular precursors. The patient was treated with pyridoxine, erythromycin, and corticotropin. Recovery was complete within ten days. As no other medicinal treatment had been given, this agranulocytosis was presumed to be due to one or more of the antituberculous agents. However, subsequent patch, intradermal, and oral tests failed to provoke a recurrence of the granulopenia, and it was eventually possible to administer all three agents in full dosage.

Comment

It is only in the past decade that prolonged chemotherapy has been established as the basic treatment of pulmonary tuberculosis, reducing resection and collapse therapy to a relatively minor supporting role. Even the value of bed rest, once the main pillar of treatment, has been called into question. Now, with the erstwhile complexities of treatment reduced to the relatively simple formula of establishing and maintaining adequate chemotherapy, the tendency is toward domiciliary treatment or shorter periods of hospitalization, with early return to work. One of the factors limiting this trend is the incidence of toxic reactions, particularly to streptomycin and PAS, for as long as we have to rely on these agents most chest physicians would agree that hospitalization is desirable for the first six to twelve weeks, when the risk is greatest. Even then, responsibility for the day-to-day care of tuberculous patients is now passing out of the hands of the specialist physician at an earlier stage than ever before. The time is thus opportune for internists and general practitioners to renew their interest in this disease and know how to recognize and deal with complications of treatment should they arise.

So much has already been written about the various toxic effects which may occur with each of the standard antituberculous drugs that it is perhaps surprising to find no other series comparable to the one described in this paper in which a retrospective survey of 3,148 cases has been carried out to discover the frequency of complications of multiple-drug therapy in conventional dosage, namely 1 gm. of streptomycin daily or twice weekly, 10 to 20 gm. of PAS daily, and 200 to 300 mg. of isoniazid daily. Complications of sufficient severity to interrupt treatment occurred in 379 patients, or 12.2 per cent. These were due to streptomycin in 7.8 per cent of those treated with this drug, in 7.6 per cent of those at risk to PAS and, for isoniazid, the figure was 0.5 per cent. In 310 patients, or 9.8 per cent, the reaction was of the allergic type, usually manifested as a drug rash and fever. In this group, PAS was the greatest offender, with a 7.2 per cent incidence against 5.8 per cent with streptomycin and 0.1 per cent with isoniazid.

The generally recognized essential harmlessness of isoniazid in normal dosage has been amply confirmed by these findings, but the total incidence, and particularly the figures for allergic reactions caused by streptomycin and PAS, are generally of a higher order than one would expect from a study of the literature. McDermott (53) quoted 5 per cent of skin eruptions in patients undergoing streptomycin therapy, but most subsequent writers have suggested much lower figures. For instance, Schwartz and Moyer (54), in an analysis of 1,734 courses of chemotherapy given in their sanatorium, found an incidence of only 0.8 per cent allergic reactions to streptomycin and 2.2 per cent to PAS. The British Medical Research Council (55) reported five such episodes (4 per cent) in 115 patients treated with streptomycin and PAS, both drugs being equally to blame. More recently, Raleigh (24) mentioned that untoward reactions interrupted treatment in 10 per cent of 280 patients treated with isoniazid and PAS. In a small series of 144 cases, McLeod (11) found an incidence similar to the present results, namely, an 8 per cent incidence of hypersensitivity to both streptomycin and PAS.

No new complications of the standard antituberculous agents have been witnessed in this

series, but most of the recognized ones have been observed. Their relative frequency and other salient features have already been discussed in the case analysis and no further comment on them seems necessary. It is believed, however, that to give as complete a picture as possible, brief mention should be made of the rarer complications which have been reported and yet which did not occur in this large series. These have therefore been listed with appropriate references in table 2.

Although the term "allergic" is commonly used to describe abnormal tissue reactions in patients who, for one reason or another, have become hypersensitive to streptomycin and PAS, it should be remembered that drug allergy is imperfectly understood and that there is no proof that it is an antigen-antibody reaction. However, it seems likely that each substance can conjugate with blood protein in some way to form an antigen. Presumably, in cases of multiple hypersensitivity, a common antigenic substance is formed which evokes the formation of antibodies which are then distributed to the various tissues of the body, including the skin and mucous membranes. Whenever there is further contact with the drug or drugs, acting either as haptens or in their conjugated form, the symptoms and signs of a reaction occur. The reaction subsides rapidly if the contact ceases but is readily reinduced by very small test doses. The capacity of the patient to become sensitized is the determining factor, *not* the dosage employed. While actual prevention is not possible, serious ill effects can be minimized by rapid recognition of the hypersensitivity state and, in all but the exceptional case, desensitization will overcome the difficulty, permitting treatment to be continued.

TABLE 2

RARE REACTIONS DESCRIBED IN THE LITERATURE BUT NOT SEEN IN THIS SERIES

Streptomycin	
Bronchial asthma and herpes simplex	Mechetti (56)
Encephalopathy	Marsh (57), Cavenagh (58)
Aplastic anemia	Goede and Palm (59), Walters (60)
Fiedler's myocarditis	Chatterjee and Thakre (61)
PAS	
Renal failure	Owen (62)
Polyneuritis	Brun, Viellier, and Kalb (63), Bulley (64)
Hypokalemia	Cayley (65)
	Crofton, French, and Sandler (66)
Auto-immune hemolytic anemia	McGibbon, Loughbridge, Hourihane, and Boyd (67)
Isoniazid	
Hepatitis	Randolph and Joseph (68)
	Gellis and Murphy (69)
Urinary complications	Blennerhassett (70)
Endocrine disturbances	Beyer (71)
Gynecomastia	Buttero, Bassoli, and Romeo (72)

In general, the picture presented by purely toxic drug reactions is quite distinct although, in the individual case, differentiation may be difficult or even impossible. The onset tends to be insidious, recovery slow and sometimes incomplete, and further administration of the offending drug is rarely possible. Here, the common causes are high dosage (especially if prolonged), contamination by degradation products, and the coexistence of any condition likely to cause abnormally high amounts of drug in the blood by interfering with the metabolism and excretion of the drug. If appropriate precautions are taken, the risk of the development of toxic phenomena can be greatly reduced. Thus, great care must be exercised with all three antituberculous drugs in the presence of renal or liver disease, and the dosage should be reduced to avoid excessive accumulation in the blood and tissues. In patients older than middle age, streptomycin, if given daily, should be restricted to as short a course as seems reasonable from a therapeutic viewpoint and, if prolonged past the sixth week of treatment, it is best given only a few times weekly, along with daily doses of isoniazid and PAS. The preparations of PAS should always be of recent manufacture and, if prolonged treatment is contemplated, high dosage should be avoided. With isoniazid, if the upper limit of dosage is limited to 5 mg. per kg. of body weight per day, toxicity need hardly be considered in normal subjects.

SUMMARY

A retrospective survey is described of the drug reactions which occurred in 3,148 patients given antituberculous chemotherapy between

1953 and 1959 in two hospitals in South Worcestershire. The occurrence of 384 reactions led to interruption of treatment in 379 patients (12.2 per cent). Of this total, 310 (9.8 per cent) were of the allergic and 74 (2.4 per cent) of the toxic variety. Of those treated with streptomycin, 7.8 per cent were affected: 7.6 per cent of those treated with para-aminosalicylic acid (PAS), and only 0.5 per cent of those treated with isoniazid.

These findings justify the conclusion that toxicity to streptomycin and PAS is one of the main problems in the chemotherapy of tuberculosis today and, for this reason, it is desirable that all patients be under close inpatient supervision during the early weeks of treatment when the risk is greatest.

Sumario

Complicaciones en la Quimioterapia de la Tuberculosis

Se describe un estudio retrospectivo de las reacciones medicamentosas que hubo en 3,148 enfermos que recibieron quimioterapia antituberculosa entre 1953 y 1959 en dos hospitales de South Worcestershire. La aparición de 384 reacciones conjudo a interrupciones del tratamiento en 379 enfermos (12.2 por ciento). De este total, 310 (9.8 por ciento) eran de la variedad alérgica y 74 (2.4 por ciento) de la tóxica. De los tratados con estreptomicina, se afectaron 7.8 por ciento: 7.6 por ciento de los tratados con ácido para-aminosalicílico (PAS) y sólo 0.5 por ciento de los tratados con isoniacida.

Justifican estos hallazgos la conclusión de que la toxicidad de la estreptomicina y del PAS es hoy día uno de los principales riesgos que rodean a la quimioterapia de la tuberculosis, y por esta razón conviene mantener a todos los enfermos bajo cuidadosa vigilancia en el hospital durante las primeras semanas del tratamiento cuando el peligro es mayor.

Resume

Complications au cours de la chimiothérapie de la tuberculose

L'auteur décrit une étude rétrospective des réactions aux médicaments qui se produisirent chez 3.148 malades à qui on donna une chimiothérapie antituberculeuse entre 1953 et 1959 dans deux hôpitaux du sud du Worcestershire. L'apparition de 384 réactions conduisit à l'interruption du traitement chez 379 malades (12,2%). Sur ce total, 310 (9,8%) appartinrent au groupe allergique et 74 (2,4%) au groupe toxique. Parmi ceux traités par la streptomycine, 7,8% furent affectés; 7,6% de ceux traités par l'acide paraaminosalicylique (PAS), et seulement 0,5% de ceux traités par l'isoniazide.

Ces données justifient la conclusion que la toxicité de la streptomycine et du PAS est aujourd'hui un des principaux dangers dans la chimiothérapie de la tuberculose, et, pour cette raison, il est souhaitable que les malades soient sous une surveillance serrée et à l'hôpital pendant les premières semaines de traitement, quand le danger est le plus grand.

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ON THE PERSISTENCE OF TUBERCULIN HYPERSENSITIVITY IN GUINEA PIGS MADE SENSITIVE PASSIVELY[1, 2]

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(Received for publication August 1, 1960)

Introduction

In 1945 Chase showed that delayed tuberculin hypersensitivity can be transferred from sensitized donor guinea pigs to normal recipient animals by means of peritoneal exudate cells, as well as by cells from spleens and lymph nodes (1). The passively transferred sensitivity, as judged by skin tests, lasted on the average of four to six days before reverting to negative. This work of Chase was substantiated by various investigators (2–4). The mechanism of this phenomenon has not yet been fully explained, although various explanations have been proposed, such as the release of sensitivity by the donor cells after transfer (2). In 1955, Metaxas and Metaxas-Buehler (5) concluded from their experiments that, in the recipient, the donor cells might be responsible for the positive skin test reaction by direct interaction with the antigen following migration to the test site during this early post-transfer period.

Similar studies in humans were carried out by Lawrence (6) who distinguished between two definite types of responses in recipients with regard to the immediate post-transfer period. Grading positive skin reactions from 1-plus to 4-plus, Lawrence found that one group of recipients would develop maximal sensitivity during the immediate post-transfer period up to approximately the eighth day. Then the sensitivity would either diminish gradually upon retesting or continue at a high level for a prolonged period of time. The second group differed from the first in that a low level of sensitivity developed initially. This lasted six to eight weeks and was followed by a sudden rise to a maximal level. As in the first group, the sensitivity would then continue at a high level or gradually decline. When dealing with the hypersensitivity of contact dermatitis, Epstein and Kligman (7) observed similar responses in humans, as did Chase (8) in guinea pigs. Working with the tuberculin-type of hypersensitivity in guinea pig recipients, Metaxas and Metaxas-Buehler (5) reported that there was a rise and fall in the passively transferred sensitivity over a four- to five-day period. This was followed by a secondary rise in skin reactivity occurring near the fifth to seventh days post transfer. They suggested that this second period of elevated skin sensitivity might be the result of active sensitization due to repeated skin testing.

It was decided to investigate the ability of passively sensitized guinea pigs to develop the prolonged or reappearing hypersensitive response in the absence of skin testing. To do this the hypersensitive state could be followed by examining the recipient's macrophages in tissue culture. It might also be determined whether or not, in passively transferred animals, any constant relationship existed between hypersensitivity as measured by skin tests and hypersensitivity as measured in tissue culture.

For this purpose experiments were set up using guinea pigs in pairs for each pool of sensitized cells to be transferred. One animal was not skin tested until the end of the experiment, but peritoneal exudate cells were removed periodically and examined in tissue culture for reactivity to tuberculin. Hypersensitivity in the other recipient was investigated by both skin test and tissue culture methods.

Materials and Methods

Balanced salt solution: Hanks balanced salt solution (9) was prepared, concentrated ten times, and stored after sterilization by filtration. Or, alternately, sterile Hanks solution, commercially obtained, was used.

Bicarbonate solution: To adjust and maintain the pH of other solutions, a 1.4 per cent sodium bicarbonate solution was prepared. After adding

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[2] This study was supported by Grant No. E-2270 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

0.002 per cent phenol red as an indicator, it was autoclaved at 115°C. for ten minutes. When used in the preparation of tissue culture medium, the solution was saturated with carbon dioxide aseptically just prior to its use.

Gelatin solution: A 10 per cent stock solution of gelatin was prepared according to the recommendation of Chase (10). This stock solution was added to Hanks solution to yield a final concentration of 0.125 per cent.

Washing fluid: Hanks solution (10× concentrated) was diluted with sterile distilled water, and sufficient 10 per cent gelatin solution was added to yield a final concentration of 0.125 per cent. The pH was adjusted to 7.4–7.6 with bicarbonate solution. Sometimes 1 per cent normal guinea pig or horse serum was substituted for the gelatin. To prevent clotting of exudate cells, heparin[3] was added. This solution was used to wash out peritoneal cavities and also to receive the spleens immediately after extirpation.

Suspending fluid: To Hanks solution 4 to 10 per cent normal horse or guinea pig serum was added. The pH was adjusted with bicarbonate solution to approximately 7.4. After washing by centrifugation, the cells were gently resuspended in this solution.

Purified protein derivative (PPD): For skin testing guinea pigs by the intradermal technique, a solution of commercial PPD[4] was used, containing 0.02 γ (first-test strength), 0.1 γ or 0.2 γ (intermediate-test strength), or 5.0 γ (second-test strength) of PPD/0.1 ml. of saline. Skin tests were carried out with first or intermediate strength on donor animals, whereas recipient animals were tested with second-strength PPD.

In order to test cells in tissue culture for tuberculin sensitivity, a solution of PPD was prepared by dissolving a weighed amount of PPD powder[5] in the required amount of Hanks solution containing 30 per cent guinea pig serum. The sterile stock solution contained 1.5 mg. of PPD per ml. and was kept frozen until used.

Three lots of PPD powder were used. The activity of each lot was confirmed by skin testing a normal and a BCG-vaccinated guinea pig and by testing concentrations ranging from 25 to 50 γ per ml. against known normal and sensitive cells in tissue culture.

Normal serum: Groups of normal guinea pigs were bled every three or four weeks. The sterile serum was pooled and kept in the frozen state until needed.

Normal horse serum was obtained (from Microbiological Associates, Inc.) and kept frozen in small amounts until used.

Antimicrobial solution: A stock solution was prepared in saline containing 5,000 units of penicillin, 5,000 γ of streptomycin, and 3,000 units of nystatin per ml. The amount added to the tissue culture medium was such as to yield a final concentration per ml. of 50 units of penicillin, 50 γ of streptomycin, and 30 units of nystatin.

Tissue culture medium: The maintenance medium consisted of Hanks solution containing 25 to 30 per cent normal horse or guinea pig serum, 15 per cent bicarbonate solution (carbon dioxide saturated), and 1 per cent antimicrobial solution. To each tissue culture flask 1.5 ml. of this medium were added.

Guinea pigs: Healthy, male albino guinea pigs, weighing from 250 to 500 gm., were obtained from various sources. The majority of animals were not tuberculin skin tested prior to being used as recipients. All of the guinea pigs were regarded as tuberculin negative, based on the experience that, of a considerable number of animals from all sources which were tested with PPD before use, none gave a positive skin test. Autopsies were performed on all recipients which died during the course of the experiment as well as on a number of donors which were eliminated before being used. Macroscopic examinations were uniformly negative for any evidence of tuberculous lesions. Aliquots of homogenized tissues derived from spleen, lung, or lymph nodes, upon culture, yielded the same negative results, and occasional histologic sections of these organs upon microscopic examination uniformly failed to reveal tuberculous lesions.

During the experiments a considerable number of animals showed evidence of streptococcal infections, latent or clinical. As a rule, vaccinated donors were eliminated from the experiment when they showed symptoms of active streptococcal infection. On the other hand, recipients were treated with antimicrobials (penicillin, streptomycin, tetracycline) and with surgical incision of abscesses when indicated.

Sensitization of guinea pigs: (*a*) Living vaccine. Male albino guinea pigs were rendered sensitive to PPD by a single, subcutaneous injection into the nape of the neck of 5 or 10 mg. of BCG.[6]

(*b*) Heat-killed organisms in Freund's adjuvant. Fifteen to 20 mg. of heat-killed tubercle bacilli in Freund's adjuvant were given in divided doses subcutaneously into the nape of the neck and intramuscularly into one or both thighs. This vaccine was prepared from an H37Rv strain obtained from the Trudeau Sanatorium, Saranac Lake, New York, and grown in Dubos liquid medium. The organisms were harvested after ten- to fourteen-days' growth, were heated for forty minutes at 70°C. to kill them, and were resuspended in saline. The ratio of Freund's adjuvant to antigen suspension was either 1:1 or 3:2 by volume. To assure sterility, random samples of the finished vaccine were cul-

[3] 1:20,000, Liquaemin sodium, Organon, Inc.

[4] Sharp and Dohme or Parke, Davis and Co.

[5] The PPD powder was generously supplied by Merck-Sharp & Dohme, West Point, Pennsylvania. This powder contained no additives, such as lactose, generally included in the lyophilized pellets used for skin testing.

[6] Supplied by the Henry Phipps Institute, Philadelphia, Pennsylvania.

tured in various media: Dubos liquid medium, Petragnani medium, Löwenstein-Jensen medium, blood agar, and nutrient broth.

Approximately twenty-five to thirty-five days after vaccination, skin sensitivity of the guinea pigs was determined by the intracutaneous injection of 0.1 ml. first-strength PPD, using normal saline at a control site. Occasionally doubtful results were checked by a second skin test with intermediate-strength PPD. Animals showing an area of induration of less than 10 mm. in diameter were not employed as donors except in isolated cases, for instance, experiment No. 17. Most sensitized animals were used as donors within a definite time period, varying from the thirty-fifth to the forty-fifth days post vaccination; but on occasion donors were used as late as ninety-two days after sensitization.

Passive transfer technique: The passive transfer of tuberculin hypersensitivity was accomplished by the technique described by Chase (1).

Peritoneal exudates: Exudates were induced by intraperitoneal injection of 5 to 30 ml. of sterile mineral oil. Two to three days later the exudates were collected in washing fluid. Exudates from several animals were pooled and, after removal of the oily layer, the cells were concentrated by light centrifugation (150–200 × g for five to fifteen minutes). The sediment was washed several times in washing fluid and finally suspended in approximately 50 per cent normal guinea pig serum in Hanks solution. Sodium bicarbonate was added to adjust the pH to approximately 7.0 to 7.4.

Samples were removed for cell counts. Differential counts showed an average range of 50 to 75 per cent macrophages, 25 to 45 per cent lymphocytes, 2 to 10 per cent polymorphonuclears, and 1 per cent eosinophils. In experiments in which BCG vaccine had been the sensitizing agent, aliquots of the final cell suspension were cultured on Petragnani, Löwenstein-Jensen, and Dubos media to detect the presence of living mycobacteria which might be carried over and cause active sensitization in the recipient animals. Smears were made from all transfer suspensions derived from H37Rv-vaccinated donors, and occasionally from those collected from BCG-vaccinated animals. The smears were stained by the Ziehl-Neelsen technique and examined for acid-fast organisms.

Splenic cell suspensions: Several spleens, from donor animals, were minced and passed through a fine wire mesh. Thereafter the suspension was treated in the same manner as the peritoneal exudates.

Transfer suspensions: In earlier experiments only peritoneal exudate cells were transferred. In later experiments the peritoneal exudates and splenic cell suspensions were pooled. The procedure described for peritoneal exudates was then carried out on the combined suspension. Each recipient animal received, on the average, 4 to 5 ml. of transfer suspension containing a total of 600,000,000 to 800,000,000 leukocytes, the extremes ranging from 300,000,000 to 1,700,000,000 cells per recipient.

The donor cells were injected into a normal recipient animal (average weight 600 to 750 gm.), frequently while under light pentobarbital sodium (Nembutal®) anesthesia. In most instances the cells were given intracardially, although in a few cases the intraperitoneal route was chosen.

Abdominal paracentesis: At intervals following the transfer of sensitive cells to recipients, macrophages of peritoneal exudates from these recipient animals were examined in tissue culture to determine their sensitivity to PPD. The exudates were obtained by intraperitoneal injection of approximately 5.0 ml. of mineral oil. Two or three days later a sterile trocar and cannula were inserted into the lower left quadrant of the abdominal cavity. The trocar was then removed, and 15 to 20 ml. of plain heparinized Hanks solution were injected as washing fluid. The abdomen was lightly massaged for a few seconds and the exudate collected through the cannula. In the majority of cases this procedure was carried out under light Nembutal anesthesia (25 mg. per kg. of body weight) and could be repeated later on the same animal without ill effects. After removal of the oil layer, the cells were immediately placed in tissue culture, providing the cell count was sufficient. Otherwise the suspension was concentrated by slow centrifugation (150 to 200 × g) and the cells were then resuspended in the appropriate amount of suspending fluid.

Tissue culture procedure: In general the technique described by Kapral and Stinebring (11) was adapted for use in these experiments. Both pooled exudate cells from donor animals and exudate cells obtained from individual recipients were examined in tissue culture. In both instances the tissue culture procedure was the same. A total of about 6,000,000 leukocytes in a volume of 1 to 2 ml. was introduced into each Porter flask. The cells were allowed to settle onto the bottom during a twenty- to forty-minute incubation period. If necessary, they were then washed with Hanks solution containing 4 per cent serum before the suspending fluid was replaced by maintenance medium. Purified protein derivative was added to experimental flasks immediately following the addition of medium. Bicarbonate solution was used as needed to maintain a pH of 7.2–7.6. In some experiments, approximately 1 ml. of medium was removed and replaced by fresh medium about every other day during the first ten days. However, PPD was added only once at the beginning of the experiment. Earlier experiments had indicated that, for the hypersensitivity reaction to develop, a one-hour contact between sensitive cells and PPD was sufficient (12). Flasks were examined microscopically approximately every day for evidence of cellular injury.

Tissue culture and skin reactions: Tissue culture results were recorded as positive (+), negative (−), or weakly positive (±). Reactions were regarded as positive in accordance with criteria described by Kapral (11). Therefore, a positive reaction represented a recipient's macrophages which, in the presence of PPD, exhibited the

tendency to round up and become progressively vacuolated until they spontaneously floated off the bottom of the flask. As controls, cells set up in exactly the same way, except in the absence of PPD, behaved like macrophages from normal guinea pigs and did not exhibit this reaction. Cells exposed to PPD were always compared with similar aliquots maintained in the absence of PPD in order to determine whether or not a specific reaction was occurring.

Skin reactions were read at twenty-four and forty-eight hours after application. The size of the tuberculin reaction was recorded as observed at twenty-four hours after injection of PPD. Diameters of redness and induration were measured in millimeters, and the amount of induration was estimated by comparing the thickness of the folded reaction area with the thickness of a fold of adjoining normal skin. In most cases a control site was injected with 0.1 ml. of saline since it served as diluent for the PPD tablets.

Results

The occurrence of prolonged passive sensitivity in recipients without early skin testing: The macrophages were collected for examination *in vitro* from one to three times following passive transfer, and 12 animals listed in table 1 (*7B*, *9A*, *10A*, *12A*, *12B*, *13A*, *13B*, *14A*, *14B*, *15A*, *15B*, and *17*) gave positive tissue culture responses before being skin tested for the first time. Of these, recipients *15A* and *15B* showed positive reactions to PPD in tissue culture more than six months after being transferred with cells from positive donor animals. The other animals were PPD-sensitive by *in vitro* tests at one and one half to almost five months' time without prior skin testing.

In comparison, the first skin test applied and giving a positive reaction was taken as another criterion for prolonged passively induced hypersensitivity irrespective of previous tissue culture results. Recipients *7B*, *9A*, *9B*, *10A*, *11*, *12B*, *13B*, and *17* are examples of the prolonged persistence of allergy from this point of view. These animals showed positive reactions three to eight months after passive sensitization. Therefore, it is unnecessary to do repeated skin testing with PPD or OT in order to obtain evidence of prolonged hypersensitivity in passively transferred animals.

Further perusal of table 1 will show that the allergy of guinea pigs *10A* and *17* to PPD had persisted longer than indicated above, but the continuation of the sensitivity was beyond the time of the first skin test. However, animals not skin tested at all in some cases (such as *14A* and *14B*) showed persistence of sensitivity for as long or longer periods than some which were skin tested.

The possibility of transfer of tubercle bacilli to the recipient via donor cells: To rule out the possibility that live BCG could be carried by the exudate cells about to be transferred, approximately 5 per cent of each exudate suspension was cultured. These cultures never yielded growth of mycobacteria. It is generally recognized that it is possible to detect by culture the presence of a very few organisms of *Mycobacterium tuberculosis* and that, under favorable conditions, the presence of one organism is sufficient to induce colony growth (13). In the case of donor animals made sensitive with killed H37Rv, acid-fast smears of aliquots of the cells to be transferred failed to reveal the presence of mycobacteria, as did the smears made from transfer suspensions of BCG-vaccinated animals. However, this method of detecting mycobacteria is, of course, relatively insensitive.

Natural infection of the recipient guinea pigs as a cause of the prolonged hypersensitive state seems highly unlikely because of the complete lack of evidence of mycobacterial infection in these animals based on the observations outlined in Materials and Methods.

The participation of donor vs. recipient cells in the recipient's expression of sensitivity: It seems reasonable to assume that mineral oil, when used in the recipient as a stimulant for peritoneal exudates, does not distinguish between recipient and donor cells and does not selectively elicit an exudation of one in favor of the other. Presumably, availability of donor and recipient cells would determine their relative numbers in any oil-induced exudate. Therefore, any exudate collected from a recipient after passive sensitization is likely to consist mainly of recipient cells, particularly when the transfer cells have been introduced intracardially rather than intraperitoneally. Also, the longer the time lapse between passive sensitization and exudate stimulation, the better the likelihood that an increasing proportion of the infused cells has been destroyed and that the exudate consists largely of the recipient's own cells.

Macrophages from passively sensitized guinea pigs which reacted to PPD in tissue culture did so in a manner different from those of animals actively sensitized with BCG or H37Rv. In the former, the onset of the reaction in tissue culture did not become evident until the fifth to

TABLE 1

SKIN TEST AND TISSUE CULTURE DATA

Recipient	Vaccine Used in Donors	Time in Days*	Tissue Culture Results	PPD Skin Test Results†	
				Reaction Size in Mm.‡	Interpretation
1	BCG	0		11 × 12 − 1	+
		1		15 × 22 − 1	+
		2		10 × 11 − 1	+
		5		10 × 10 − 1	+
		16		11 × 12 − 1	+
2	BCG	0		5 × 5 − 1	+
		3		Not recorded	+
		29		17 × 20 − 1	+
		46		Not recorded	+
		49	+		
		53		0	−
3	BCG	0		6 × 10 − 1	+
		1		10 × 11 − 1	+
		23		15 × 15 − 1.5	+
4	BCG	0		0	−
		1		5 × 5 − 0.5	+
		45	+		
5	BCG	520		10 × 11 − 0.5	+
6§	BCG	1		8 × 10 − 1	+
		2	+		
		3		0	−
7A	BCG	7	+		
		29	−		
		145	−		
		326		0	−
7B	BCG	7	−	−	−
		29	−	−	−
		145	±		
		149		6 × 6 − 1	+
		326		0	−
8A	BCG	5	−	Not recorded	+
8B	BCG	5	+		
9A	BCG	112	±	11 × 15 − 0.3	+
9B	BCG	112	−		
		279		Not recorded	+
10A	BCG	83	±	6 × 6 − 0.5	+
		258		14 × 14 − 1.0	+
10B	BCG	83	−		
		258		0	−
11	BCG	253		18 × 18 − 1.5	+
12A	BCG	57	±		
12B	BCG	57	+		
		112		15 × 15 − 1.5	+
13A	BCG	44	±		
13B	BCG	44	+		
		109		10 × 11 − 1	+
14A	H37Rv	144	+		
14B	H37Rv	99	+		
		144	+		
15A	H37Rv	112	+		
		197	+		
		211		0	−
15B	H37Rv	112	+		
		197	+		
		211		0	−
16	H37Rv	1		15 × 20 − 1	+
		66	+		
17	BCG	38	+		
		94	+		
		98		18 × 18 − 1	+
		129	+		
18	BCG	0		0	−
		1		0	−
		2		0	−
		3		0	−
		30	−	0	−
		60	−	0	−
		148		0	−
19	BCG	0		0	−
		1		0	−
		2		0	−
		3		0	−
		30	−	0	−
		60	−	0	−
		180		0	−
		360		0	−
		496		0	−

Italicized numbers represent animals which were skin tested once with PPD before being used as recipients.

* Days between injection of transferred cells and the indicated procedure, either paracentesis or skin test.

† 0.1 ml. second-strength PPD intracutaneously.

‡ The first 2 figures indicate redness; the third, the elevation of the induration.

§ 4.0 ml. of transfer suspension injected intracardially; 2.0 ml., intraperitoneally.

seventh days and frequently took longer to reach a peak, whereas the cells from actively sensitized guinea pigs responded to PPD in about two to three days. This slow response observed with "recipient cells" *in vitro* was independent of the time interval between passive transfer and subsequent performance of the paracentesis. Furthermore, the degree of reactivity of these cells, although varying from animal to animal, remained constant in each recipient, often for a considerable period of time when serial exudates were tested, as for example with recipient *No. 17*. This indicated that at a time exceeding the expected survival period of donor cells in the recipient, the recipient's own cells probably were responsible for the positive reaction. On the other hand, shortly after passive transfer, when the presence of a certain percentage of donor cells in a recipient's peritoneal exudate would seem more probable, some factor may have been released by the donor cells and taken up by the recipient cells so that the cell mixture appeared to be uniformly sensitive. It would seem immaterial whether such a postulated transfer occurred in the body of the recipient or in the tissue culture. Whether this consistent difference in reactivity between donor and recipient macrophages in tissue culture can be attributed to a difference in degree of sensitivity or to a basic difference in the quality of the sensitivity needs further study.

To illustrate this further, recipient *No. 6* received two-thirds of a transfer suspension intracardially and one-third intraperitoneally. Exudate cells were removed two days later and examined in tissue culture. In contrast, recipient *No. 8B* received its entire dose of donor cells by the intracardial route. The paracentesis performed on the latter recipient was at five days instead of two. In both tissue culture studies, the macrophages reacted positively to PPD with no significant difference in the over-all development and intensity of the responses. In the first case one would expect a greater proportion of donor cells in the exudate than in the second case. Yet this was probably not so, since there was no detectable difference in reactivity.

To investigate whether or not it was possible to detect in tissue culture a small proportion of sensitive cells among normal cells, several tissue culture experiments were set up in which macrophages from normal guinea pigs were mixed in varying proportions with macrophages from skin-test positive, actively sensitized animals. Even when the mixed population contained only 25 per cent of sensitive cells, the entire population reacted to PPD. How much further the percentage of sensitive cells could be lowered without an obvious change in response to PPD needs to be investigated. The cell mixtures were always compared with control flasks containing either all sensitive or all normal cells, and also with control mixtures to which PPD had not been added. Mixtures of cells in other ratios with increasing percentage of sensitive cells gave similar results. In addition, cell mixtures were made from animals showing strong, moderate, and weak skin sensitivity to PPD. Again, when the reactions in these cell cultures were compared with each other, no differences in the proportion of cells reacting could be found. Neither the presence of a comparatively low percentage of sensitive cells, nor the fact that in some cases the sensitive cells were obtained from weakly sensitive donors as judged by skin test, seemed to cause any appreciable delay in the tissue culture reaction.

Relationship between tissue culture results and skin test: The experimental results presented here indicate a rather constant qualitative agreement between skin test and tissue culture results, when both tests were carried out at approximately the same time. Exceptions are recipient *No. 2* (tissue culture test on the forty-ninth day and skin test on the fifty-third day) and recipient *No. 8A* (skin test and tissue culture carried out on the fifth day). However, in order to draw a definite conclusion, a considerably greater number of animals would have to be tested.

Miscellaneous observations: Occasionally it was found that a recipient animal would give a positive skin test once or several times, and when tested subsequently would show a negative result. This is demonstrated by recipient *No. 2*, which on days 1, 3, 29, and 46 post transfer gave positive skin reactions, but on the fifty-third day reacted negatively. Recipient *No. 7B* was negative with respect to both tissue culture and skin test on the seventh and twenty-ninth days, but showed a weakly positive result in tissue culture one hundred and forty-five days after transfer. On the one hundred and forty-ninth day two skin sites on recipient *No. 7B* gave reactions of $5 \times 5 \times 0.5$ mm. and $6 \times 6 \times 0.5$ mm. and 2 negative (saline) control sites. On the three hun-

dred and twenty-sixth day skin tests on the same animal were again negative. It is interesting to note that in recipient *No. 2* a period of repeated skin tests with positive results was followed by a negative response one week later. The repeated contact with PPD apparently did not enhance the duration of hypersensitivity as measured by skin test. Recipient *No. 7B* reacted similarly, only the period of positive response developed considerably later before returning to negative.

There was considerable variation in the response of individual recipients of a pair which received aliquots of a pool of cells, as for example recipient pairs (*A* and *B*), *Nos. 7* to *10*, inclusive. This variation extended to all phases of the passively transferred sensitivity. Thus, one recipient of a pair might become sensitized, whereas the other one would remain negative with respect to both skin test and tissue culture whenever tested. Or, sensitivity might appear early in one recipient, but much later in the other. Also, paired recipients tended to differ with respect to duration and fluctuation of sensitivity.

Discussion

Metaxas and Metaxas-Buehler (5) suggested, from their observations in recipients, that the skin response obtained at five to seven days post transfer was the result of active sensitization from tuberculin skin testing. The tuberculin used in their experiment, prepared in the same manner as Old Tuberculin, apparently could sensitize normal animals by repeated injection; or, perhaps, the material was somewhat toxic. However, it is generally agreed that "Old Tuberculin," because of the heating to which it is subjected in the course of its preparation, is nonantigenic (14). This also applies to PPD which is derived from a heated culture filtrate (15). The PPD used in the present experiments was not antigenic. Nonsensitive animals repeatedly injected with PPD failed to develop even slight skin reactions to this material. In table 1, 2 animals are listed (*Nos. 18* and *19*) which were skin tested seven and nine times, respectively, but remained negative in their response to intradermal PPD. It was noted, however, that some lots of commercial PPD diluent would elicit a slightly positive, tuberculin-like reaction in both sensitive and normal animals. This reaction was, therefore, of a toxic nature. Thus, possible differences between tuberculin products used by Metaxas and Metaxas-Buehler and those used in the experiments discussed here may offer a partial explanation for some of the discrepancies in results. There is no doubt, however, that a positive response in tissue culture can be obtained without active sensitization by skin testing.

Although active sensitization as a result of skin tests can be excluded, on the one hand, the possibility of active sensitization caused by antigenic material carried across by donor macrophages cannot be completely eliminated. Experimental evidence, such as the culturing of exudates from BCG-sensitized donors and the negative autopsy reports, contradicts this. However, culturing of an aliquot may not necessarily detect a single or few mycobacteria present in the total exudate.

The question which remains to be answered is whether or not active sensitization might be caused by antigen in soluble form carried across by donor macrophages. If this is so, it would be reasonable to expect that preparations of killed macrophages or fractions thereof could transfer tuberculin hypersensitivity. Extracts of sonically vibrated cells or cells treated by repeated freezing and thawing have been ineffective in guinea pigs (1, 4, 16, 17), although such preparations have transferred tuberculin sensitivity in humans (6, 18–20) when injected subcutaneously. One exception was reported by Cummings and co-workers (21), who successfully transferred tuberculin sensitivity in guinea pigs with cells prepared by sonic vibration.

Assuming that active sensitization plays no role in the experiments described here, what mechanism could explain then the presence of sensitivity in the recipient over such a prolonged period of time?

The possibility exists that an antibody-like substance is transferred with the donor cells and becomes firmly attached to recipient cells. Under conditions of relatively tight fixation to cells, it would not be readily metabolized or eliminated by the recipient's system and, therefore, would persist for a much longer period of time than the circulating type of antibody.

On the other hand, the donor cells conceivably may transfer to the recipient's own cells an intrinsic mechanism which will permit in the recipient, in the absence of antigen, the continued production of the factor responsible for pro-

longed hypersensitivity. The unit transferred must include a mechanism for conveying information regarding specificity.

Summary

Normal guinea pigs were passively sensitized to tuberculin by transfer of peritoneal exudate and splenic cells from actively sensitized donors. At intervals, paracentesis was performed on the recipients, and the mononuclear cells were examined for sensitivity to PPD in tissue culture. Skin tests were performed at various times.

Prolonged sensitivity can be demonstrated without bringing the recipient into contact with the antigen by skin testing. The possibility of active sensitization by antigen transfer to the recipient via donor cells appears to be unlikely, but cannot be excluded entirely. Hypersensitivity seems to be transferred to the recipient's mononuclear cells, so that they themselves become reactive to PPD in tissue culture. The duration of passively transferred hypersensitivity may exceed several months.

Sumario

Sobre la Persistencia de la Hipersensibilidad a la Tuberculina en los Cobayos Sensibilizados Pasivamente

Se sensibilizó pasivamente a la tuberculina a cobayos normales por medio del pase de exudado peritoneal y células esplénicas procedentes de donantes sensibilizados activamente. A ciertos intervalos, se ejecutó la paracéntesis en los recibidores, y se examinaron los mononucleares en cuanto a sensibilidad al DPP en histocultivos. Se verificaron cutirreacciones en varias ocasiones.

Puede descubrirse la existencia de sensibilidad prolongada sin poner al recibidor en contacto con el antígeno por medio de cutirreaccionando. La posible sensibilización activa por el pase de antígeno al recibidor por vía de las células del donante parece improbable, pero no puede ser excluída del todo. Parece que se traspasa la hipersensibilidad a los mononucleares del recibidor, de modo que éstos a su vez se vuelven reactores al DPP en histocultivos. La duración de la hipersensibilidad traspasada pasivamente puede exceder de varios meses.

Resume

De la persistance de l'hypersensibilité à la tuberculine chez des cobayes rendus sensibles passivement

Des cobayes normaux furent sensibilisés passivement à la tuberculine par transfert d'exsudat péritonéal et de cellules spléniques de donneurs sensibilisés activement. Des paracentèses furent effectuées par intervalles chez les sujets, et les cellules mononucléaires furent examinées au point de vue sensibilité à la PPD dans des cultures de tissus. On pratiqua des cutiréactions à différentes époques.

On put démontrer une sensibilité prolongée sans amener le sujet en contact avec l'antigène lors de la réaction cutanée. La possibilité d'une sensibilisation active par transfert d'antigène chez le sujet, au moyen des cellules du donneur semble être invraisemblable, mais ne peut être complètement exclue. L'hypersensibilité semble être transmise aux cellules mononucléaires du sujet, de telle façon qu'elles deviennent elles-même capables de réagir vis à vis de la PPD dans une culture de tissus. La durée de l'hypersensibilité transmise passivement peut dépasser plusieurs mois.

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STUDY ON THE PASSIVE HEMAGGLUTINATION REACTION BY THE PHOSPHATIDE OF M. TUBERCULOSIS[1]

I. The Reaction and Its Specificity

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(Received for publication March 11, 1960)

INTRODUCTION

The phosphatide of tubercle bacilli has generally been considered incapable of sensitizing erythrocytes to hemagglutination or hemolysis in the presence of tuberculous serum. In the laboratory of the present study it was found, however, that the phosphatide also possessed an erythrocyte-sensitizing capacity as high as or higher than that of the tuberculin polysaccharide generally used in the Middlebrook-Dubos hemagglutination test. In fact, sheep erythrocytes appropriately sensitized with saline emulsions of phosphatide are led to specific agglutination in the presence of tuberculous serum and, on addition of adequate amounts of complement, lysis of the agglutinated erythrocytes takes place. In addition, the antigen-antibody system involved in the phosphatide hemagglutination was found to be completely independent of that in the polysaccharide hemagglutination. The results of the study were outlined in previous papers (1, 2). The present communication deals with the details of the study.

MATERIALS AND METHODS

Antigens

Phosphatide of strain Nakano (phosphatide 1): According to the method of Nègre (3), slightly modified cultures of human tubercle bacilli of the Nakano strain, aged eight weeks on Sauton medium, were heat-killed, dried, and preliminarily extracted with a sufficient amount of acetone. The residue was then extracted three times at approximately 45°C. for five to six hours with ten volumes of methanol. The methanol extracts were combined, Seitz-filtered, and evaporated to dryness *in vacuo*. The yellowish-white product thus obtained was stored in a refrigerator until used. This sample will be referred to hereafter as *phosphatide 1*.

Phosphatide of BCG (phosphatide 2): This sample was obtained from acetone-killed cultures of BCG, aged eight weeks on Sauton medium, by the method described above except that, after having been dried, it was treated with boiling acetone to eliminate acetone-soluble fats. This sample will be referred to hereafter as *phosphatide 2*.

Phosphatide of strain Nakano (phosphatide 3): This sample was extracted from heat-killed cultures of the Nakano strain cultivated for eight weeks on Sauton medium according to the method of Anderson (4). It will be referred to hereafter as *phosphatide 3*.

Tuberculin polysaccharide: Use was made of three different preparations of tuberculin polysaccharide, which will be referred to hereafter as *polysaccharides 1, 2,* and *3*, respectively. *Polysaccharide 1* was prepared from an unheated culture filtrate of the Nakano strain aged eight weeks on Sauton medium. This is a fraction precipitable at 90 per cent methanol, after removal of a fraction precipitable at 30 per cent methanol. *Polysaccharides 2* and *3* were also obtained from an unheated culture filtrate of strain H37Rv of the same age and on the same medium. These are fractions precipitable at 80 per cent and 95 per cent methanol, respectively, after removal of a fraction precipitable at 50 per cent methanol. Tuberculin polysaccharide fractions precipitable at low concentrations of methanol correspond to "polysaccharide II" of Seibert and associates (5). They contain almost no nitrogen and are serologically inactive *in vitro*. For isolation of the polysaccharide fractions, the culture filtrates had previously been deproteinized by addition of trichloracetic acid. The fractions obtained were purified by repeated dissolution in distilled water, centrifugation, and reprecipitation with methanol.

Tuberculin: For comparison, use was made of a Sauton tuberculin prepared from a culture filtrate of human tubercle bacilli, strain Aoyama B, aged eight weeks on Sauton medium. For serologic use, an aliquot of the tuberculin was dialyzed in a cellophane bag against running tap water for two days, followed by dialysis against distilled water for one day.

The chemical properties of the antigens used are presented in table 1.

Preparation of Antigen Solutions

For sensitizing erythrocytes, the polysaccharide preparations were dissolved in saline to the desired concentrations.

The phosphatide preparations were used as

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TABLE 1

CHEMICAL PROPERTIES OF THE ANTIGENS

Antigen	Nitrogen*	Phosphor†	Molish's Test	Orcin-HCl
	per cent			
Polysaccharide 1	trace	0.53	+++‡	+‡
Polysaccharide 2	0.22	0.15	+++	+
Polysaccharide 3	1.00	0.20	+++	+
Phosphatide 1	0.29	2.60	++	±
Phosphatide 2	1.47	2.68	++	±
Phosphatide 3	0.15	1.97	++	±

* According to the technique of Kjeldahl.
† According to the technique of Allen.
‡ These marks indicate the degrees of reaction.
+++ = very strongly positive
++ = strongly positive
+ = positive
± = doubtful reaction
The Biuret, Millon, and xanthoprotein reactions were negative for all of the antigens.

saline emulsions at a concentration of 0.5 mg. per ml. To obtain phosphatide emulsions, the phosphatides were first dissolved in methanol at a concentration of 2 mg. per ml. An aliquot of the methanol solution was poured drop by drop into four volumes of saline which were under constant agitation by means of a magnetic stirrer. The glass container with the emulsion was then brought onto a water bath at approximately 50°C., and the methanol in the emulsion was rapidly evaporated by aeration with an electric fan. (The fan was turned off when there was no longer an odor of methanol.) After having been adjusted with distilled water to the original volume of saline, the emulsion was ready to be used. Merthiolate to a final concentration of 1:10,000 was added to all of the antigen solutions or emulsions, and they were stored in a refrigerator at 4°C. until used. In this way, the antigens are usable for at least two weeks without significant alterations.

Tuberculous Serum

Rabbits weighing between 2 and 3 kg. were immunized with the following materials (each group comprised 2 to 3 rabbits):

Bacilli BA: human tubercle bacilli of the strain Aoyama B, aged five weeks on Sauton medium, killed by immersion in acetone for twenty-four hours, and dried. Nitrogen content: 4.56 per cent.

Residue RC: bacilli BA successively exhausted with acetone, methanol, and chloroform. The exhaustion was repeated three to four times for five to seven hours at approximately 50°C., with each solvent five times the volume of the residue. The bacilli in this residue were still acid fast. Nitrogen content: 6.25 per cent.

Residue RHA: residue RC deprived of its bound lipids by treatment with 1 per cent hydrochloric acid alcohol for five hours at approximately 50°C. This residue no longer contained acid-fast bacilli, and consisted exclusively of cyanophilic amorphous debris. Nitrogen content: 8.25 per cent.

Living BCG: bacilli obtained from a culture aged five weeks on Sauton medium.

Living human tubercle bacilli: bacilli of the strain Nakano, aged five weeks on Sauton medium.

Heat-killed human tubercle bacilli: the same material as above, killed by heating in an autoclave.

All of the materials were suspended in saline and injected through the ear vein. The injected doses of *bacilli BA, residue RC,* and *residue RHA* were controlled so that they contained equal amounts of nitrogen. They were injected eight times at weekly intervals, totaling 12 mg. for *bacilli BA,* 8.25 mg. for *residue RC,* and 6.72 mg. for *residue RHA.* The other bacillary suspensions were injected three times at weekly intervals in doses of 10 mg. each time.

The animals were all bled two weeks after the last injection. The sera obtained were pooled, Seitz-filtered, and, after having been inactivated at 56°C. for thirty minutes, stored in a refrigerator at 4°C. until used. Before using, the sera were absorbed with normal sheep erythrocytes which were to be used for the hemagglutination reaction. Finally, 0.2 ml. of each serum specimen was mixed with 1.0 ml. of 10 per cent erythrocyte saline suspension, left at room temperature for ten minutes, and centrifuged. This was repeated twice for each aliquot of serum.

Erythrocytes

Sheep erythrocytes were used. Sheep blood was collected aseptically in a sterile flask containing glass beads for defibrination. After defibrinization, the blood was mixed with an equal volume of modified Alsever's solution, as recommended by Boyden and Sorkin (6), which had been autoclaved at 10 pounds for fifteen minutes, and stored at 4°C. until used. At the beginning of each day, an aliquot of the blood was taken and the erythrocytes were washed three times with saline and then resuspended in saline to 2.5 per cent. The stored blood was not used for three weeks after collection.

Serologic Tests

Hemagglutination test: In preliminary experiments it was confirmed that, for all of the antigens comprising the phosphatides, a concentration of 0.5 mg. per ml. of saline and a ratio of antigen solution to packed cells of 40:1 were adequate. These conditions were adopted throughout the present experiments.

In the sensitization of erythrocytes, 4 ml. of antigen solution were added to each 0.1 ml. of washed packed cells. The mixture was incubated at 37°C. for two hours, after which it was washed three times with saline and then resuspended in

saline to 2.5 per cent. Serial twofold dilutions of the sera to be tested were made with saline in 0.5-ml. amounts in serologic tubes (10 × 80 mm.) to which the sensitized erythrocyte suspensions were added at the rate of 0.05 ml. each. Then the tubes were incubated at 37°C. for two hours, during which they were shaken every fifteen minutes. Thereafter, the tubes were left at room temperature overnight. The reading was made the next morning by observing the pattern formed by the cells settled at the bottom of the tubes. The controls consisted of one tube containing 2.5 per cent normal erythrocyte suspension plus the lowest dilution of the serum to be tested, and other tubes containing serial twofold dilutions of normal rabbit serum plus 2.5 per cent sensitized erythrocyte suspension.

Cross-inhibition test: Serial twofold dilutions of antigen were made in 0.5-ml. amounts in serologic tubes. To each tube was added 0.5 ml. of a definite dilution of antiserum. The tubes were incubated at 37°C. for thirty minutes. Thereafter, 0.5 ml. of 2.5 per cent erythrocyte suspension sensitized with appropriate antigen preparations was added to each tube. The remainder of the procedure was the same as in the hemagglutination test.

Results

As may be seen in table 2, sheep erythrocytes sensitized with either of the three phosphatide preparations agglutinated to a high degree in the presence of various tuberculous sera, as did those sensitized with the polysaccharide antigens. In this experiment, the erythrocytes sensitized with polysaccharide reacted with sera against *residue RC* that had been totally deprived of its free lipids. This occurred almost to the same extent as in sera obtained by injection of dead or living whole bacilli. By contrast, the phosphatide-sensitized cells exhibited almost no re-

TABLE 2

Results of Tests for Hemagglutination Reaction

Number of Sera	Immunogens*	Sheep Erythrocytes Sensitized with						
		Phosphatide			Polysaccharide			OT
		1	2	3	1	2	3	
1	BA	1,280†	1,280	2,560	640	—	—	—
2	BA	640	640	640	640	160	160	160
3	BA	5,120	2,560	5,120	640	80	160	—
4	RC	—	—	—	640	160	320	320
5	RC	—	—	20	1,280	320	640	320
6	RC	—	—	—	1,280	640	640	640
7	RHA	80	—	80	80	—	—	—
8	RHA	—	—	20	40	—	—	—
9	RHA	40	—	40	80	—	—	—
10	BCG (living)	1,280	640	1,280	1,280	640	640	640
11	BCG (living)	640	640	1,280	1,280	640	640	640
12	BCG (living)	640	640	1,280	1,280	640	640	640
14	Aoyama B (living)	320	320	320	320	160	160	320
15	Aoyama B (living)	320	160	320	640	—	640	160
16	Aoyama B (killed)	1,280	640	1,280	640	320	160	160
17	Aoyama B (killed)	320	320	640	640	320	320	640
19	Control	—	—	—	40	—	—	—
20	Control	—	—	—	20	—	—	—

* BA: whole bacilli of the human strain Aoyama B killed by immersion in acetone; RC: bacilli BA deprived of its free lipids; RHA: residue RC deprived of its bound lipids; OT: Old Tuberculin; phosphatides 1, 2, and 3: phosphatide samples; polysaccharides 1, 2, and 3: tuberculin polysaccharide samples.

† The figures under the column heads for the various samples indicate the maximal positive dilutions of the sera. — = negative reaction.

TABLE 3
RESULTS OF THE CROSS-INHIBITION TEST*

Sensitizing Antigen	Inhibiting Antigen						
	Phosphatide			OT	Polysaccharide		
	1	2	3		1	2	3
Phosphatide 1	1,024†	512	512	—	—	—	—
Phosphatide 2	1,024	128	512	—	—		
Phosphatide 3	1,024	2,048	2,048	—	—	—	—
OT	—	—	—	128	1,024		
Polysaccharide 1	—	—	—	—	16	16	16
Polysaccharide 2	—	—			128	32	16
Polysaccharide 3	—	—			512	256	256

* Serum No. 10, dilution 160 ×. Similar results were obtained with serum No. 14.
† The figures indicate the maximal inhibiting dilutions of the inhibiting antigens (1 mg. per ml).
— = no inhibition; blanks = lack of reaction.

action against these anti-*residue RC* sera. These findings indicate clearly that the formation of antibody to the phosphatide of tubercle bacilli takes place independently of that to the polysaccharide. It is also noteworthy that, when tubercle bacilli were completely defatted by treatment with 1 per cent acid alcohol (*residue RHA*), they lost almost completely their antigenicity *in vivo*. This would suggest the importance of bound lipids in the production of antibody.

It is well known that, because it is different from the phosphatides of animal or plant origin, the phosphatide of tubercle bacilli contains a relatively large amount of polysaccharide. On the other hand, in the hemagglutination test using normal erythrocytes, polysaccharides are said to be sensitizing agents. These facts involve the possibility that, in the phosphatide hemagglutination reaction, the polysaccharide moiety in the phosphatide preparations may sensitize erythrocytes to hemagglutination. However, the results of the cross-inhibition test revealed that this was not the case. The phosphatide hemagglutination was not inhibited by the tuberculin polysaccharide preparations, and vice versa, as may be seen in table 3. This indicates that there is no serologic relationship, at least between tuberculin polysaccharides and bacillary phosphatides.

DISCUSSION

It is well known that the phosphatide of tubercle bacilli is active *in vitro*. In fact, the so-called "*antigène méthylique*" of Nègre (3), consisting mainly of phosphatide, and Anderson's phosphatide (4), have been used for the complement-fixation test and the precipitin test (3). However, no report has been available on the erythrocyte-sensitizing capacity of this lipid. Gernez-Rieux and Tacquet have stated that the *antigène méthylique* did not sensitize erythrocytes to hemagglutination (7).

In the light of the present investigations, the failure of the *antigène méthylique* to sensitize erythrocytes seems to have rested in its low concentration in phosphatide. As is well known, the *antigène méthylique* is a mere methanol extract of tubercle bacilli previously extracted with acetone. For preparation of the antigen, methanol is added to the acetone-treated bacilli at the rate of 1 ml. to 0.1 gm. of bacilli. For serologic use, the methanol extract is usually diluted twenty fold by saline (8). In this way, the phosphatide content of the saline suspension is roughly calculated to be 0.1 to 0.15 mg. per ml. at the maximum on the assumption that the total amount of bacillary phosphatide is extracted. In the experiments which appear in the following paper (9), it will be seen that 0.1 mg. of phosphatide per ml. is not sufficient for the sensitization of sheep erythrocytes. Moreover, it should be borne in mind that a mere methanol extract of tubercle bacilli still contains a considerable amount of acetone-soluble fat and chloroform-soluble wax. The antigenicity of these substances has not as yet been established.

In the present study three different samples of

phosphatide prepared by different methods were used with success: they all exhibited high hemagglutination reactions, almost to the same degree. None of them, however, reacted with serum obtained by injection with bacillary residue totally deprived of its free lipids (*residue RC*). By contrast, erythrocytes sensitized with the polysaccharide preparations reacted to the same degree as did sera obtained by injection of whole bacilli. This indicates that the antigen-antibody reaction participating in the phosphatide hemagglutination reaction is independent of that participating in the polysaccharide hemagglutination. The results of the cross-inhibition test were compatible with this finding. Thus, the specificity of the phosphatide hemagglutination reaction is believed to have been indisputably demonstrated. Lysis of phosphatide-sensitized erythrocytes on addition of complement will be considered in the following paper (9).

Summary

The phosphatide of *M. tuberculosis* is capable of sensitizing normal sheep erythrocytes to hemagglutination in the presence of tuberculous serum. The antigen-antibody system participating in the phosphatide hemagglutination is completely independent of that participating in the polysaccharide hemagglutination.

Sumario

Estudio de la Reacción de Hemaglutinación Pasiva por la Fosfátide del M. Tuberculosis. I. Ejecución de la Reacción y su Especificidad

La fosfátide del *M. tuberculosis* es capaz de sensibilizar los eritrocitos normales de oveja a hemaglutinación si hay suero tuberculoso presente. El sistema de antígeno-anticuerpo que participa en la hemaglutinación con fosfátide es absolutamente independiente del que participa en la hemaglutinación con polisacárido.

Resume

Etude sur la réaction d'hémagglutination passive par le phosphatide du M. tuberculosis. I. Réalisation de la réaction et sa spécificité

Le phosphatide du *M. tuberculosis* est capable de sensibiliser les érythrocytes normaux de mouton à l'hémagglutination, en présence de sérum tuberculeux. Le système antigène-anticorps participant dans l'hémagglutination au phosphatide est complètement indépendant de celui qui participe à l'hémagglutination au polysaccharide.

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STUDY ON THE PASSIVE HEMAGGLUTINATION REACTION BY THE PHOSPHATIDE OF M. TUBERCULOSIS[1]

II. Conditions of the Reaction

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(Received for publication March 11, 1960)

INTRODUCTION

In the preceding paper (1), it was reported that the phosphatide of tubercle bacilli, isolated either by Anderson's method or Nègre's method, was capable of sensitizing normal sheep erythrocytes to hemagglutination in the presence of tuberculous sera in the same fashion as polysaccharide preparations isolated from tuberculins. It was also pointed out that the phosphatide hemagglutination reaction belongs to another antigen-antibody system entirely distinct from that concerned in the polysaccharide hemagglutination reaction. In the experiments which are to be reported in the present paper, efforts were made to find the best conditions for demonstrating the reaction by examining the technique for preparation of phosphatide antigens, sensitization of erythrocytes, et cetera. During the course of the experiments, it was found that the phosphatide hemagglutination reaction was converted to the hemolysis reaction on addition of complement. For comparison, the complement-fixation and precipitation tests were performed with the same phosphatide antigens.

MATERIALS AND METHODS

Antigens

The phosphatide sample used in the present study was extracted with methanol from a mixture of different cultures of tubercle bacilli (strains BCG, H37Rv, and Nakano) grown for eight weeks on Sauton medium, killed by immersion in acetone, and dried. About 60 gm. of the bacilli were first subjected to extraction with 600 ml. of acetone. The extraction was made twice at approximately 40°C. with the same volume of acetone for three hours each time. After having been dried, the residue was extracted twice at the same temperature with the same volume of methanol for five hours each time. The methanol extracts were combined, Seitz-filtered, and evaporated in a nitrogen atmosphere to 200 ml. to which two volumes of acetone were added. The precipitate formed was collected by centrifugation, redissolved in warm methanol, and again centrifuged to eliminate impurities. Reprecipitation by addition of acetone was followed by drying *in vacuo* (yield: about 1.7 gm., P:2.2 per cent).[2] An aliquot of the yellowish-white product was finally dissolved in methanol at a concentration of 2.0 mg. per ml., and stored in a refrigerator at 4°C. until used. Before using, the methanol solution was warmed in a water bath to dissolve the precipitates formed during storage.

Preparation of Antigen Solutions

Since tuberculophosphatides are water-insoluble, they must be emulsified in saline from methanol solution for use in serologic testing. It was believed that differences in the procedures for preparation might affect the eventual serologic activities of the antigen emulsions. Therefore, in the present study two different methods of preparation were compared.

Drop method: Several methods were compared in which the antigen was added to the saline or, conversely, saline to the antigen, under mechanical or manual agitation. Of the methods, the following was used principally:

As is described in the preceding paper (1), a definite quantity of saline was first placed in a flat-bottomed glass container. To the saline, which was under constant agitation by means of a magnetic stirrer, a definite amount of phosphatide methanol solution was added drop by drop so that the desired final concentrations of phosphatide could be obtained. The container was then brought onto a water bath at approximately 50°C., and the methanol contained in the emulsion was rapidly evaporated by aeration with an electric fan. The emulsion was adjusted with distilled water to the original volume of saline. In the drop method, a syringe furnished with a ½ hypodermic needle[3] was used to make for homogeneous conditions, except as otherwise stated in the text. This

[1] From the Research Institute for Tuberculosis, Hokkaido University, Kita-12, Nishi-5, Sapporo, Japan.

[2] It was confirmed afterward that this product contained a fair amount of serologically inactive, chloroform-insoluble, waxlike substance, but it was satisfactorily used for serologic purposes. The phosphatide hemagglutination reaction conferred by this phosphatide sample was by no means inhibited by the tuberculin polysaccharide preparations used in the preceding study (1).

[3] 25-mm. long, 0.6-mm. in diameter.

method will be referred to as the antigen-to-saline drop method.

Mixing methods: A definite amount of saline taken in a test tube (15 × 150 mm.) was added at once to a definite amount of methanol solution of phosphatide taken in another test tube. The resulting emulsion was mixed rapidly by repeated decantation into the alternate tube. The last tube containing the emulsion was put into an incubator at 37°C. for thirty minutes. Following this, the emulsion was decanted into a flat-bottomed glass container which was then brought onto a water bath at about 50°C. The methanol present in the emulsion was eliminated in the same manner as mentioned above.

Immune Serum

Tuberculous rabbit serum obtained by injections with living BCG bacilli was used (sera 21, 27, and 28). The sera obtained were Seitz-filtered, distributed in small ampoules and, after having been inactivated at 56°C. for thirty minutes, stored in a refrigerator at 4°C. until used. Before using, 0.2 ml.-aliquots of serum were absorbed with 1 ml. of 10 per cent erythrocyte suspension to be used for hemagglutination tests.

Erythrocytes

Sheep erythrocytes were used. The procedure for collection and storage of blood and preparation of erythrocyte suspensions was the same as described in the preceding paper (1).

Diluent

Except as otherwise stated in the text, phosphate-buffer saline at pH 7.2 was used; this was made by adding to the saline 1/10 volume of phosphate buffer at pH 7.2.

Serologic Procedures

Hemagglutination test: Hemagglutination tests were performed, varying the concentrations of antigen and erythrocyte suspension and the time of sensitization of erythrocytes. The conditions applied will be described for each experiment. Otherwise, the tests were conducted according to the following procedure: 0.05 ml. of packed erythrocytes was mixed with 2.0 ml. of 0.5 mg. of phosphatide emulsion per ml., and incubated at 37°C. for two hours, during which the tubes containing the mixture were shaken every thirty minutes. Thereafter, the sensitized erythrocytes were washed twice with saline and resuspended in saline to 2.5 per cent. Serial twofold dilutions of the serum were made in 0.5-ml. amounts in rows of small test tubes (10 × 80 mm.). To each test tube 0.05 ml. of the sensitized erythrocyte suspension was then added, and the tubes were incubated at 37°C. for two hours. The tubes were also shaken every thirty minutes. They were read after having been left overnight at room temperature. The reading was made by observing the sedimentation patterns of the erythrocytes settled at the bottom of the tubes. The following mixtures served as controls: (*a*) sensitized erythrocytes plus diluent, and (*b*) nonsensitized erythrocytes plus the lowest dilution of serum to be tested.

Hemolysis test: Hemagglutination tests were first conducted as described above in several rows of small test tubes, using saline as the diluent, and 0.25-ml. serial dilutions of test serum. After the reading was made, 0.1 ml. of complement dilutions containing, respectively, 3, 4, 5, 6, 8, and 10 complement units per 0.1 ml., as measured according to the method of Ogata (2), were added to the tubes row by row. The tubes were placed in a water bath at 37°C. for thirty minutes. The reading for hemolysis was made immediately after, and the intensity of hemolysis was graded according to the following signs: O: no hemolysis, 1: partial hemolysis, 2: fair hemolysis, 3: complete hemolysis.

Complement-fixation test: According to the checkerboard method of Ogata (2), both the antigen emulsion and the test sera were serially diluted.

Precipitation test: The ring method was applied in this test. As was done in the complement-fixation test, serial dilutions of antigen were tested against serial dilutions of test serum.

Results

Drop method: According to the methods described in the preceding section, the following five different antigen emulsions were prepared and tested for hemagglutination reaction. The phosphatide concentration for each was 0.5 mg. per ml. Saline was used as the diluent.

Emulsion a: prepared by the antigen-to-saline drop method, using a syringe, and under constant agitation by means of a magnetic stirrer (about 300 drops per 1 ml. of methanol solution).

Emulsion b: prepared by the same method as (*a*), except that a 1-ml. pipette was used for the drop method (about 45 drops per 1 ml. of methanol solution).

Emulsion c: prepared by the same method as (*b*), but under manual agitation instead of using a magnetic stirrer.

Emulsion d: prepared by first placing methanol solution in drops on the surface of the saline, and then shaking with a magnetic stirrer.

Emulsion e: prepared by dropping the saline into the methanol solution with a syringe, and agitating with a magnetic stirrer. (Nègre applied a similar method for preparing aqueous emulsions from his *antigène méthylique* (3).)

As may be seen in table 1, preparations *a*, *b*, and *c* exhibited the same constant hemagglutination titers toward all the sera tested, while preparation *d* lacked stability in hemosensitization.

TABLE 1

HEMOSENSITIZING ACTIVITIES OF VARIOUS ANTIGENS

Antigen	Serum Titers of Antibodies		
	21	27	28
	Maximal positive dilutions of serum		
a	640	640	320
b	640	640	320
c	640	640	320
d	640	640	—
e	—	—*	—

* The dashes in all of the tables represent negative reactions.

TABLE 2

DIFFERENCE IN ACTIVITY OF ANTIGENS ACCORDING TO DIFFERENT METHODS OF EVAPORATING METHANOL

Evaporating Method	Serum Titers of Antibodies		
	21	27	28
	Maximal positive dilutions of serum		
Water bath	640	640	320
Desiccator	320	320	—

Preparation *e* was found to be incapable of sensitizing erythrocytes.

Comparing the method of preparation by which the antigen was added dropwise to the saline, as in preparation *a* (the antigen-to-saline drop method), and the method by which, conversely, saline was added to the antigen, a remarkable difference in stability was noticed between the two emulsion samples. The emulsions prepared by the former method presented only slight opacity and opalescence; they were very stable and yielded no precipitates after standing at room temperature for several days. By contrast, emulsions prepared by the latter method were cloudy, showed no opalescence, and were unstable. They yielded precipitates readily after a short period at room temperature or after centrifugation at 2,000 r.p.m. for five minutes.

In order to obtain information about the influence of temperature on the eventual erythrocyte-sensitizing capacity of the phosphatide, the emulsions were prepared according to the former method at 20°C., at room temperature (about 25°C.), and at 40°C., respectively. No significant difference was noticed between these preparations.

In the next experiment, the procedure of eliminating methanol from the emulsions was examined. A freshly prepared sample of emulsion was divided into equal moieties. For one moiety, the methanol was evaporated conventionally on a water bath at about 50°C. by aeration with an electric fan. The methanol in the other moiety was evaporated in a small desiccator by means of an air pump. As may be seen in table 2, the former procedure was found favorable for serologic tests.

Mixing method: It was possible to prepare active emulsions by the mixing method, but they were generally somewhat unstable and less active in erythrocyte sensitization than emulsions prepared by the antigen-to-saline drop method. Moreover, the emulsions showed variations from one preparation to another. The mixing of the saline and the antigen had to be done as quickly as possible. Slow mixing sometimes resulted in inactivity of the emulsions. In this case also, methanol had to be eliminated from the emulsions, as in the case of the antigen-to-saline drop method; the presence of methanol in concentrations of more than 20 per cent resulted in spontaneous hemolysis of sensitized erythrocytes.

Antigen Concentrations and Concentrations of Sensitized Erythrocytes

According to the antigen-to-saline drop method and the mixing method, phosphatide emulsions ranging from 2 mg. per ml. to 0.01 mg. per ml. were prepared. To each of the emulsions erythrocytes were added serially in 1/10, 1/20, 1/40, 1/80, and 1/160 quantities. After having been incubated, washed, and resuspended by the conventional methods, the sensitized erythrocytes were tested, respectively, for hemagglutination. As may be seen in tables 3 and 4 in the emulsions prepared by the antigen-to-saline drop method, constant hemagglutination reaction was observed for all concentrations of erythrocytes in a wide range of antigen concentrations from 2 mg. per ml. to 0.25 mg. per ml. By contrast, for the emulsions prepared by the mixing method, the reaction was constant only

for the erythrocyte concentrations of less than 1/20 volume in a narrow range of antigen concentrations from 0.4 mg. per ml. to 0.2 mg. per ml. It was noticeable that, in the latter case, 1/10 volume of erythrocytes could not be satisfactorily sensitized in this range of antigen concentrations. Similar tendencies were observed for the other sera tested.

Influence of Hydrogen Ion Concentration

In order to examine the influence of the pH of the diluent on the phosphatide hemagglutination reaction, 4 different samples of buffered saline at pH 5.0, 6.0, 7.0, and 8.0 were prepared by adding to the saline 1/10 volume of M/15 phosphate buffer with a respective pH value. Hemagglutination tests were performed, using these 4 different saline samples and ordinary saline without buffer for all of the procedures required. The emulsions were prepared by the antigen-to-saline drop method so that they contained 0.5 mg. of phosphatide per ml. As may be seen in table 5, spontaneous hemolysis of the sensitized erythrocytes resulted at pH 5.0. At pH 6.0, the reactions were unstable. Maximal titers were obtained with salines at pH 7.0 and 8.0 and with ordinary saline without buffer.

Time and Temperature for Sensitization and Reaction

Erythrocytes were sensitized at 37°C. for 30, 60, and 120 minutes, respectively. They were then put into reaction with the sera at 37°C. for 30, 60, and 120 minutes, respectively. It was found from this experiment that both sensitization and reaction took place satisfactorily at 37°C. for 30 minutes, and the reaction mixture showed maximal titers after this period at 37°C. No hemagglutination occurred after sensitization at room temperature (about 25°C.) for 15 minutes, followed by reaction under the same condition. Maximal titers were obtained, however, after sensitization and reaction at room temperature for 30 to 60 minutes, respectively.

Reading of Tests

In the phosphatide hemagglutination reaction, the erythrocyte aggregates which formed on the bottom of the test tubes appeared rather soft when compared with those observed in the case of the polysaccharide hemagglutination reac-

TABLE 3

RELATIONSHIP BETWEEN ANTIGEN AND ERYTHROCYTE CONCENTRATIONS

1. Results of the Antigen-to-Saline Method with Serum 28

Antigen Concentration	Erythrocyte Concentration (Volume)				
	1/10	1/20	1/40	1/80	1/160
mg. per ml.	*Maximal positive dilutions of serum*				
2.0	320	320	320	320	320
1.0	320	320	320	320	320
0.5	320	320	320	320	320
0.25	320	320	320	320	320
0.1	160	320	320	320	320
0.05	—	—	80	160	160
0.01	—	—	—	—	—

TABLE 4

RELATIONSHIP BETWEEN ANTIGEN AND ERYTHROCYTE CONCENTRATIONS

2. Results of the Mixing Method with Serum 28

Antigen Concentration	Erythrocyte Concentration (Volume)				
	1/10	1/20	1/40	1/80	1/160
mg. per ml.	*Maximal positive dilutions of serum*				
2.0	—	—	—	—	—
1.0	—	—	—	80	160
0.5	—	—	160	320	320
0.4	80	320	320	320	320
0.3	80	320	320	320	320
0.25	80	320	320	320	320
0.2	80	320	320	320	320
0.1	—	—	160	320	320
0.05	—	—	—	80	80
0.01	—	—	—	—	—

TABLE 5

INFLUENCE OF pH OF DILUENT ON HEMAGGLUTINATION

Diluent	pH	Serum Titers of Antibodies		
		21	27	28
		Maximal positive dilutions of serum		
Buffered saline	5.0	hemolysis	hemolysis	hemolysis
	6.0	—	320	—
	7.0	640	640	320
	8.0	640	640	320
Ordinary saline	9.0	640	640	320

TABLE 6

STABILITY OF ANTIGENS: RESULTS WITH SERUM 28
(Similar Results Were Obtained with Serum 27)

Antigen Prepared by	Temperature	Conservation Period in Days											
		0	1	2	3	5	7	10	14	21	28	35	42
		Maximal positive dilutions of serum											
Antigen-to-saline method	40°C.	320	320	320	320	320	320	320	320	320	320	160	—
	Room	320	320	320	160	160	160	160	160	160	160	160	—
Mixing method	40°C.	320	320	320	320	320	320	160	—	—	—	—	—
	Room	320	—	—	—	—	—	—	—	—	—	—	—

tion. By gently shaking the tubes, the aggregates generally dispersed again into a homogeneous suspension, not in clumps.[4] Consequently, the reading had to be made by observing the sedimentation patterns of only the settled erythrocytes. However, no change in titers was noticed when the tubes were shaken, left at room temperature, and later read again.

Stability of Antigen Emulsion

An 0.5-mg. per ml. emulsion and an 0.25-mg. per ml. emulsion were prepared by the antigen-to-saline drop method and the mixing method, respectively. Each emulsion was then divided into equal moieties. One moiety was stored in a refrigerator at 4°C., and the other was left at room temperature (15° to 30°C.). Aliquots were taken from the emulsions 0, 1, 2, 3, 5, 7, and 10 days and 2, 3, 4, 5, and 6 weeks after preparation, and tested for their hemagglutination ability. As is shown in table 6, the emulsion prepared by the antigen-to-saline drop method was found to be far more stable than that prepared by the mixing method. It is noteworthy that at 4°C. the former preparation maintained its maximal activity for as long as four weeks after preparation. By contrast, the latter preparation was susceptible to spontaneous agglutination both in a refrigerator and at room temperature.

[4] In this connection, it has since been found that phosphatide antibodies are very susceptible to heating at 56°C. for 30 minutes. With this treatment, almost 50 to 80 per cent of their activity is lost. When EDTA (disodium ethylenediaminetetraäcetate) is used instead of heating at 56°C. for 30 minutes in inactivating test serum, intense hemagglutination reactions are obtained with much higher titers than occur ordinarily. In this case, the resulting hemagglutinates are adherent; by shaking the tubes, they generally rise in clumps.

Hemolysis Reaction

For testing the hemolysis reaction, the test sera were diluted serially in 0.25-ml. amounts instead of 0.5-ml. amounts as were used for the hemagglutination tests. This was done because the hemolysis reaction was easy to read when the erythrocyte suspension to be used was appropriately thick. As may be seen in table 7, as the amounts of complement added were increased, the hemolysis titers increased. However, the addition of 10 units of complement resulted in hemolysis of the sensitized erythrocytes without the presence of tuberculous serum. The hemolysis titers were found to be one-half to four times the hemagglutination titers, according to the amounts of complement added.

Complement-Fixation and Precipitation Tests

For both the complement-fixation and precipitation tests, an 0.5-mg. per ml. emulsion prepared by the antigen-to-saline drop method was used as the starting antigen. In the complement-fixation test, the antigen titers ranged from 4 or 8 to 512, as may be seen in table 8, but the antibody titers were one-quarter to one-eighth of the hemagglutination titers. The results of the precipitation test are presented in table 9. The antigen titers were 32 for all sera, and the antibody titers ranged from 16 to 32, according to the sera, and were almost 1/20 of the hemagglutination titers.

DISCUSSION

The present investigations emphasize the importance of the stability of phosphatide saline

TABLE 7

HEMAGGLUTINATION AND HEMOLYSIS REACTIONS: RESULTS WITH SERUM 27
(Similar Results Were Obtained with Sera 21 and 28)

Test	Units of Complement Added per 0.1 Ml.	Serum Dilution: Reciprocals of the Dilution Titers										Reading
		20	40	80	160	320	640	1,280	2,560	C_1*	C_2	
Hemagglutination												320
Hemolysis	3	3†	2	2	2	1	0	0	0	0	0	160
	4	3	3	3	3	2	0	0	0	0	0	320
	5	3	3	3	3	2	2	0	0	0	0	640
	6	3	3	3	3	3	3	2	0	0	0	1,280
	8	3	3	3	3	3	3	2	0	0	0	1,280
	10	3	3	3	3	3	3	2	1	0	1	

* C_1 : control for nonsensitized erythrocytes toward immune serum (1/20)
C_2 : control for sensitized erythrocytes toward saline
† 3: complete hemolysis; 2: fair hemolysis; 1: partial hemolysis; 0: no hemolysis

TABLE 8

COMPLEMENT-FIXATION TEST: RESULTS WITH SERUM 27
(Results of Equal Tendencies Were Obtained with Sera 21 and 28)

Serum Dilution	Antigen Dilution: Reciprocals of the Dilution Titers										
	2	4	8	16	32	64	128	256	512	1,024	CS*
20	3	1	0	0	0	0	0	0	1	3	3
40	3	2	0	0	0	0	0	0	1	3	3
80	3	3	2	1	0	0	0	0	1	3	3
160	3	3	3	3	3	3	1	2	3	3	3
320	3	3	3	3	3	3	3	3	3	3	3
CA†	3	3	3	3	3	3	3	3	3	3	3

* CS: control for serum
† CA: control for antigen

emulsions in the realization of the hemagglutination reaction. It is noteworthy that the stability differs considerably according to the different phosphatide emulsions prepared by the different methods and that unstable emulsions have little activity in sensitizing normal sheep erythrocytes. Nègre (3) postulated a technique for preparing saline emulsion from his *antigène méthylique* in which saline was added to the antigen drop by drop, and then more rapidly, while under constant agitation. According to Nègre, the emulsions thus prepared were utilizable for the complement-fixation test with tuberculous serum. In the present study, however, phosphatide emulsions prepared according to this method were found to be unstable and did not sensitize erythrocytes (preparation *e*). This is probably due to the fact that, when compared with the *antigène méthylique*, the phosphatide methanol solution used in the present study was too thick (2.0 mg. per ml.) to give a dispersion fine enough to sensitize the erythrocytes. In any case, this type of method cannot be used for the preparation of phosphatide emulsions for use in the hemagglutination test.

In the kaolin agglutination test for syphilitic serum in Japan, saline emulsions of cardiolipin are prepared by briskly adding saline to the antigen, and then shaking vigorously (5). In the present study, phosphatide emulsions prepared by this mixing method were also found to be active in sensitizing erythrocytes. But this

TABLE 9

PRECIPITATION TEST WITH SERUM 27
(Similar Results Were Obtained with Sera 21 and 28)

Antigen Dilution	Serum Dilution							
	Reciprocals of the Dilution Titers							
	1	2	4	8	16	32	64	128
1	+	+	+	+	+	+	±	−
2	+	+	+	+	+	+	±	−
4	+	+	+	+	+	+	±	−
8	+	+	+	+	+	+	±	−
16	+	+	+	+	+	+	±	−
32	±	±	+	+	±	±	−	−
64	−	−	−	−	−	−	−	−

+ = positive reaction
± = doubtful reaction
− = negative reaction

method implies two disadvantages: that the range of the antigen concentrations optimal for sensitizing erythrocytes is narrow, and that the sensitizing activity varies from one preparation to another according to the rapidity of addition and mixing. In the writers' experience, slow motion in mixing the saline and the antigen usually resulted in low titers of the test sera.

The best method was found to be the antigen-to-saline drop method, in which the antigen was added dropwise to the saline under constant agitation by means of a magnetic stirrer. Under constant conditions for dropping and agitating, constant stable phosphatide emulsions high in erythrocyte-sensitizing activity were always obtainable. (In this laboratory, a 1.0-ml. pipette was used.) Moreover, the emulsions were stable for a long period of time at 4°C., and the range of concentrations optimal for sensitizing erythrocytes was far wider than in emulsions prepared by the mixing method. For these reasons, the antigen-to-saline drop method should be applied in the preparation of phosphatide emulsions for use in hemagglutination tests.

A comparison of the three different serologic tests—the hemagglutination, complement-fixation, and precipitation tests—using the same antigen revealed that the hemagglutination test was the most sensitive, as it was capable of detecting far smaller doses of antibodies. It is conceivably advantageous that, upon addition of appropriate amounts of complement, the phosphatide hemagglutination reaction can be converted to the hemolysis reaction, giving still higher antibody titers. Moreover, as compared with the complement-fixation test, the procedure for the passive hemagglutination reaction is far more simple. It is hoped that this phosphatide hemagglutination reaction method will be widely used for serologic studies in tuberculosis. The clinical significance of the reaction is under investigation.

SUMMARY

The antigen-to-saline drop method was found to be the best method for the preparation of antigen emulsions suitable for the passive hemagglutination reaction by the phosphatide of tubercle bacilli. Ordinary saline or buffered saline at pH 7.0 and 8.0, prepared by adding 1/10 volume of phosphate buffer at respective pH values, was found suitable for obtaining the reaction.

The sensitization of erythrocytes and the reaction of the mixtures of antigen and sensitized erythrocytes took place satisfactorily at 37°C. for thirty minutes. Of the complement-fixation, precipitation, and hemagglutination tests, the last-named gave the highest antibody titers. Upon addition of complement, the phosphatide hemagglutination reaction was converted to the hemolytic reaction, giving still higher antibody titers.

SUMARIO

Estudio de la Reacción de Hemaglutinación Pasiva por la Fosfátide del Bacilo Tuberculoso. II. Condiciones de la Reacción

La técnica de la gota de antígeno-a-solución salina resultó ser la mejor para la preparación de emulsiones antigénicas apropiadas para ejecutar la reacción de hemaglutinación pasiva por la fosfátide del bacilo tuberculoso. Para obtener la reacción, resultaron adecuadas tanto la solución salina corriente como la amortiguada a un pH de 7.0 y 8.0, preparadas agregando 1/10 por volumen de amortiguador fosfático a los respectivos valores de pH.

La sensibilización de los eritrocitos y la reacción de las mezclas de antígeno y eritrocitos sensibilizados tuvieron lugar satisfactoriamente a 37°C. durante treinta minutos. De las pruebas de fijación del complemento, precipitación y hema-

glutinación, la última mostró los mayores títulos de anticuerpo. Al agregar complemento, la reacción de hemaglutinación con fosfátide se convirtió en la reacción hemolítica, acusando títulos todavía mayores de antígeno.

Resume

Etude de la réaction d'hémagglutination passive par le phosphatide du bacille tuberculeux. II. Conditions de la réaction

La méthode de goutte à goutte d'antigène dans une solution saline a été trouvée la meilleure méthode de préparation d'émulsions d'antigènes convenables pour obtenir la réaction passive d'hémagglutination par le phosphatide du bacille tuberculeux. Une solution saline ordinaire ou une solution saline tamponnée à un pH de 7.0 et 8.0; préparée en ajoutant $\frac{1}{10}$ de volume du tampon phosphaté à des valeurs de pH correspondantes, at été trouvées convenables pour obtenir cette réaction.

La sensibilisation des érythrocytes et la réaction des mélanges d'antigènes et d'érythrocytes sensibilisés se produisit d'une façon satisfaisante à 37°C, pendant trente minutes. Parmi les réactions de fixation du complément, de précipitation et d'hémagglutination, cette dernière donna les taux les plus élevés d'anticorps. Suivant l'addition de complément, la réaction d'hémagglutination au phosphatide fut convertie en réaction hémolytique, donnant encore des taux d'anticorps plus élevés.

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Case Report

EOSINOPHILIC GRANULOMA OF THE LUNG[1]

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(Received for publication August 3, 1960)

INTRODUCTION

Eosinophilic granuloma is a disease of unknown cause characterized by the formation of a granuloma, containing chiefly eosinophils and histiocytes. The bones are most frequently involved; but the gastrointestinal tract, lymph nodes, skin, pituitary, kidneys, lungs, and other organs may be affected.

In the recent literature, many reports have appeared describing pulmonary eosinophilic granuloma or pulmonary histiocytosis X without demonstrable skeletal or visceral lesions.

It is the purpose of this paper to add an additional case in which complete pulmonary function studies have been performed and to review this disease entity.

CASE REPORT

In February, 1953, a 24-year-old white male developed an acute upper respiratory infection, and a chest roentgenogram taken at that time was said to show "some shadows." After these findings were confirmed by follow-up films he was admitted to another hospital on February 2, 1954, where physical examination was found to be unremarkable.

Laboratory studies showed a hemoglobin of 14.9 gm. per 100 ml., and a leukocyte count of 11,600 with 63 per cent neutrophils, 31 per cent lymphocytes, and 3 per cent eosinophils. The sedimentation rate was 11 mm. per hour. Blood chemical constituents were as follows: carbon dioxide, 21 mEq./l; chloride, 102 mEq./l; the total serum calcium was 11.6 mg. per 100 ml.

A roentgenogram of the chest showed an increase of the bronchovascular markings and numerous soft, small densities throughout both lung fields, more pronounced in the middle thirds (figure 1). Roentgenograms of skull, hands, and feet were normal. An electrocardiogram was normal. The patient reacted to PPD but not to histoplasmin and coccidioidin. Pulmonary function studies revealed no impairment. Lymph node and liver biopsy were normal. A lung biopsy was performed on March 30, 1954. The specimen consisted of a small segment of lung tissue from the right middle lobe, measuring 1.5 by 2 cm. It presented a number of small, slightly indurated, grayish nodules varying in size from 1 to 5 mm.

Histologic examination: The pulmonary parenchyma was infiltrated by discrete, irregular, patchy, granulomatous areas. These were made up of histiocytes with nuclei similar to a nucleus of a monocyte, and they were infiltrated with eosinophils and a few lymphocytes and plasma cells (figures 3 and 4). Some of the granulomas showed fibrosis and contained many macrophages laden with a finely granular golden-yellow pigment and fine black granules (figure 5). There were numerous eosinophils and a moderate number of lymphocytes at the periphery of these areas. Small foci were made up chiefly of monocytic histiocytes and a few eosinophils, while eosinophilic granulomas were also encountered containing few histiocytes. The alveolar septa at the periphery of the granulomatous areas were thickened by an infiltrate of eosinophils and monocytic cells. In the granulomatous lesions the alveolar spaces were collapsed and lined with hypertrophic septal cells (figure 6). The blood vessels in this location had thickened walls (figure 7). In the intervening parenchyma, many of the alveoli were filled with finely vacuolated macrophages laden with pigment. Giemsa stain for *Histoplasma capsulatum* and Gridley's stain for fungi were both negative.

Beginning in July, 1954, the patient began to note loss of weight which totaled 50 pounds by March, 1955. He was admitted to the New York Veterans Administration Hospital on March 3, 1955, because of malaise, chills, fever, left anterior chest pain, and cough productive of yellow sputum of one week's duration.

Physical examination revealed a thin, well-developed man who did not appear ill. A right thoracotomy scar and a diffuse tinea versicolor eruption on the chest were noted. There were fine rales in both bases, more on the right. A well-healed scar was noted in the left trochanteric area. The temperature was 103.2°F., the pulse rate was 100 per minute; the respirations, 28 per minute; and the blood pressure 128 systolic, 70 diastolic in mm. of mercury. Examination of the blood showed a hemoglobin of 13 gm. per 100 ml. and a leukocyte count of 14,700 cu. mm. with 61 per cent neutrophils, 32 per cent lymphocytes, 47 per cent monocytes, 2 per cent eosinophils, and 1 per cent basophils. The sedimentation rate was 15 mm. per hour. Total cholesterol was 242 mg. and cholesterol esters, 75 per cent. The chlorides were

[1] From the Pulmonary Disease Service, Veterans Administration Hospital, First Avenue at East 24th Street, New York 10, New York.

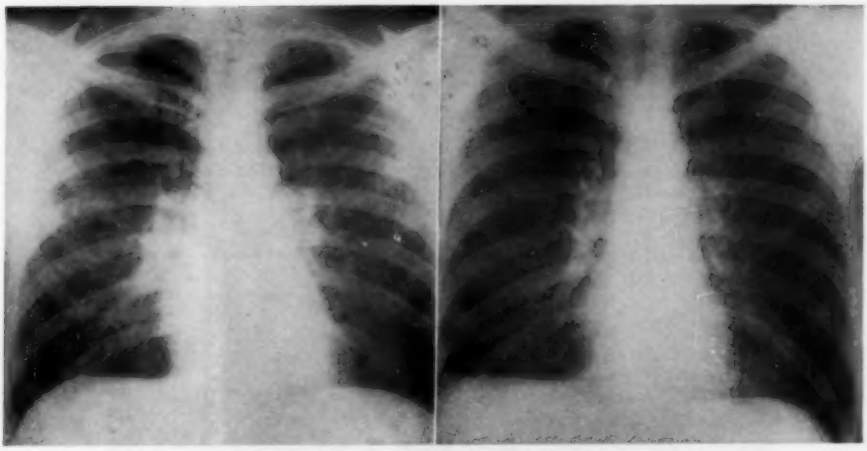

FIG. 1 (Left). Diffuse pulmonary infiltration.
FIG. 2 (Right). Partial clearing.

104.5 mEq./l, the sodium 140 mEq./l, the potassium 4.0 mEq./l. Sputum culture showed pneumococci. Blood cultures were sterile. The sputum contained no eosinophils. A roentgenogram of the chest revealed bilateral diffuse fine nodulation rather symmetrically involving chiefly the middle thirds. Comparison of the admission film with that of June 14, 1954, showed no change. A skeletal survey revealed only an old healed fracture of the left femur. The patient was treated with penicillin and on the fourth hospital day became afebrile and the pulmonary symptoms subsided. Results of pulmonary function studies done on July 14, 1955, were as follows:

	Predicted	Observed
Vital capacity	4,550	3,931
Residual volume	1,137	1,516
Total capacity	5,687	5,447
Ratio RV/TC	20%	27%
Maximal breathing capacity	140 l/min.	147 l/min.

Arterial oxygen saturation was 96 per cent when inspiring 21 per cent oxygen and dropped to 91 per cent when the patient inspired 14 per cent oxygen. This drop is within normal limits. These studies further revealed that the venous admixture was 6 per cent of cardiac output and that the oxygen diffusing capacity was 27, both of which are normal values. A rest-and-exercise study showed rather marked hyperventilation with exercise, but no significant drop in arterial oxygen saturation after one minute of standard exercise.

In view of the minimal decrease in pulmonary function, consisting essentially of a slight reduction in vital capacity, it was believed that there was no indication for any treatment. The patient was followed at intervals of six months, and his chest films showed further clearing. He was readmitted to the hospital on June 25, 1957, because of dyspnea and headaches. Physical examination and clinical laboratory studies were not remarkable. A skeletal survey again revealed no osseous involvement. Repeat pulmonary function studies disclosed improvement of the vital capacity, which was 4,592 cc. as against the figure of 3,931 cc. found in July, 1955. The rest of the pulmonary function studies showed no change. Since the patient had a history of anxiety reaction, it was thought that his complaints were due to that condition. Symptoms subsided, and he was discharged on July 24, 1957.

The patient had three more admissions for dyspnea, headache, and gastrointestinal complaints. The gastrointestinal series was found negative. His symptoms were again attributed to anxiety. At his most recent observation on July 6, 1960, his chest films showed no change from that of June 25, 1957 (see figure 2), and measurements of maximal breathing capacity and vital capacity were within normal limits.

DISCUSSION

Review of the Literature

In 1929, Finzi (1) described "a myeloma with a preponderance of eosinophilic cells" in a fifteen-year-old boy. It was not until 1940 that Lichtenstein and Jaffe (2) applied the term "eosinophilic granuloma" to the bone lesion of a four-year-old child. Histologically, the lesions were characterized by sheetlike collections of histiocytes and eosinophilic polymorphonuclear cells.

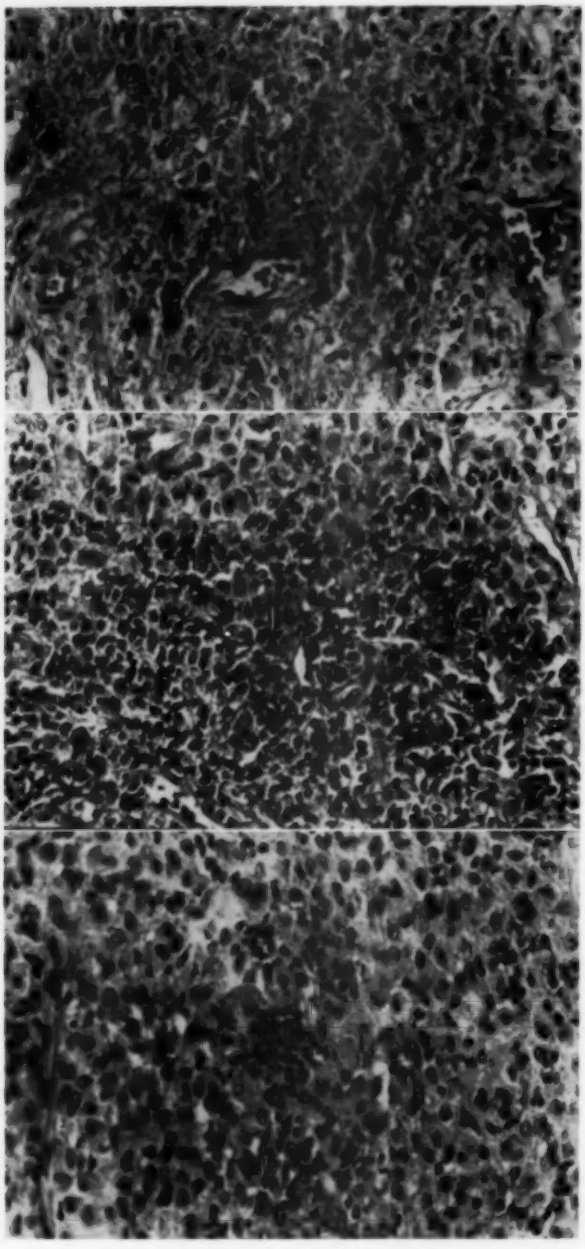

FIG. 3 (Left). Predominantly histiocytic infiltration, with small foci of eosinophils.
FIG. 4 (Center). In addition to the histiocytes there is a dense infiltration of eosinophils.
FIG. 5. A few giant cells. Much brown pigment was present in this area.

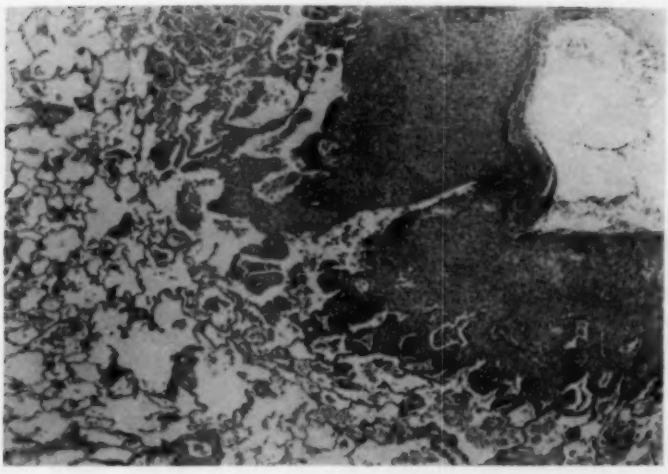

FIG. 6. Low-power microphotograph of a nodule with central cavity. Note the thickening of the septa at the periphery of the lesion, while further away the alveoli are essentially normal with slight emphysematous distention.

FIG. 7. The vessel wall is infiltrated by eosinophils.

Although Lichtenstein and Jaffe did not classify the granuloma, Farber (3) and Green and Farber (4) considered it a variant of Hand-Schüller-Christian disease and Letterer-Siwe disease. Mallory (5) agreed with Farber in this concept.

Currens and Popp (6), in 1943, first reported a case of eosinophilic granuloma of bone in which some form of pulmonary involvement was recognized, but the later paper of Engelbreth-Holm, Teilum, and Christensen (7) more fully describes this combined entity and now ranks as a classic in this field.

Lichtenstein (8), in 1953, reviewed all of the known facts and concluded that all of these syndromes are expressions of the same disease. He introduced a new term, "histiocytosis X" for eosinophilic granuloma in a localized form and used the name of Hand-Schüller-Christian disease for the chronic disseminated form and Letterer-Siwe disease for the acute or subacute disseminated form. He also speculated that, although eosinophilic granuloma may be manifested in skeletal areas, such lesions may have their histiocytic counterparts in extraskeletal tissues, becoming apparent only months or years later.

The first two cases of eosinophilic granuloma with demonstrable pulmonary involvement only, proved by lung biopsy, were reported by Farinacci, Jeffrey, and Lackey in 1951 (9). The writers, in conclusion, stated that the lesions were "like eosinophilic granuloma of the bone, the monsymptomatic form of a systemic xanthomatous disorder."

Many papers followed describing eosinophilic granuloma of the lung in association with extrapulmonary lesions, but histologic confirmation of the pulmonary pathology in association with osseous lesions was first reported by May, Garfinkle, and Dugan (10).

In surveying the English literature, it has been possible to find forty-six cases of eosinophilic granuloma of the lung proved by biopsy. In thirty-six (9, 11, 14–17, 20, 22–26, 28–31) of those cases, including the one reported in this paper, the lesion was limited to the lungs. Five (10, 12, 19) were associated with bone lesions. Six patients (10, 13, 16, 18, 27) had diabetes insipidus. One patient (21) had a clinical course and lung biopsies leading to a diagnosis of "Loeffler's pneumonia" and eosinophilic granuloma.

Grossly, the lesions consist of firm nodules which are diffusely scattered within the lung parenchyma as well as subpleurally. They vary in size from 5 mm. to 2 cm. and are usually bilateral. The margins are indistinct, without encapsulation. There is no special relation to the bronchi. On section the nodules are dark gray or tan, and no caseation is present. The pleura overlying a nodule may be puckered or dome-shaped. Blebs or cysts on the surface of the lung are frequently described, and pneumothorax is a fairly common complication. Adhesions, if present, are usually fine and not extensive. Hilar lymph nodes may be enlarged.

Microscopically, the histologic picture is one of granulomatous infiltration of alveolar septa and bronchiolar walls. The distinctive cell is the histiocyte, described as a moderately large pale cell with a centrally placed vesicular nucleus. Cell outlines are usually distinct but sometimes a syncytium is formed, and occasionally there are giant cells. Various amounts of brown pigment may be present. Foam cells have also been described, but these usually are absent. The presence of eosinophils lends additional support to the diagnosis, and these have almost invariably been described in the lesions. Occasional neutrophils, lymphocytes, or plasma cells may also be seen.

The walls of arterioles are frequently infiltrated by histiocytes and eosinophils, and such changes as intimal proliferation, fibrinoid degeneration, or fragmentation of the elastica may be present. Similar loss of elastic fibers is demonstrated in the alveolar septa and bronchioles (figure 8). The tissue reaction, in the "early" proliferative phase, appears to consist of an abundance of reticulin fibers. Later there is the formation of collagen and dense fibrous tissue. Noncaseous central necrosis occurs in some of the lesions, and bronchial communications can be demonstrated.

Although the cause of pulmonary eosinophilic granuloma remains unknown, the following causes have been considered: bacterial, mycotic and viral infection, helminthic infestation, Hodgkin's disease, berylliosis, sarcoidosis, and hypersensitivity. The patients thus far reported are predominantly males, usually in the third or fourth decades. There appears to be no geographical predilection.

The onset is insidious. Cough, productive or nonproductive, is the commonest complaint.

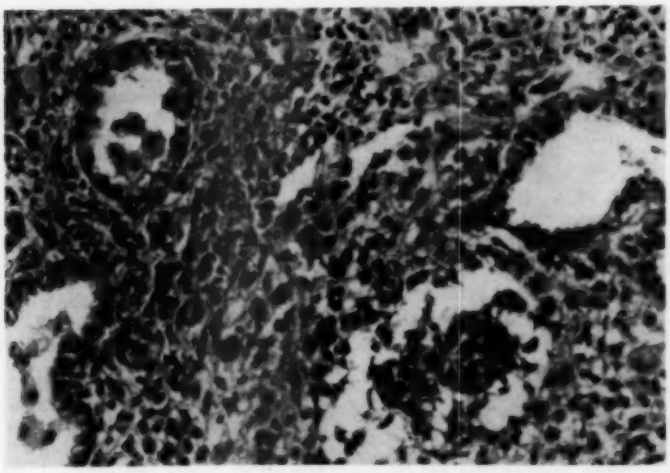

FIG. 8. Involvement of the bronchiolar walls.

Weight loss, dyspnea, fatigue, fever, malaise, and hemoptysis have been noted. Spontaneous pneumothorax, single or recurrent, due to rupture of subpleural blebs is not infrequent. Manifestations of diabetes insipidus also have been reported. Occasionally the patients are asymptomatic. Except in cases of pneumothorax, the physical examination is noncontributory. Routine laboratory findings, with the exception of a usually elevated leukocyte count and sedimentation rate, are normal. Mild peripheral eosinophilia is sometimes encountered, as well as positive coccidioidin and histoplasmin skin tests. Cultures of sputum for bacteria, when such were done, have been noncontributory.

In the patient of this report several sets of pulmonary function studies were done over a seven-year period. These studies revealed values within normal limits, except for a slightly decreased vital capacity in the second study done on July 14, 1955.

Studies of pulmonary function have been made in a number of the cases reported in the literature. Studies which could be considered adequate are available, however, in only 9 of these cases (25, 31). Most of the patients' studies have shown some reduction in the vital capacity and total capacity. Maximal breathing capacity has usually been normal, but was definitely reduced in three patients. Residual volume was elevated in six, but no abnormality of alveolar mixing was detected in these by the method of nitrogen washout. Carbon dioxide retention was not found. Oxygen saturation was usually normal, but one patient showed unsaturation at rest and another, following exercise. In the few patients listed as having cor pulmonale, it seems probable that most of the above abnormalities would have been found if adequate studies had been performed. There is some suggestion that the more severe disturbances have been noted in patients whose disease was of longer duration, but this is by no means a regular observation. The disease in a majority of patients followed for a period of years has pursued a benign course.

Roentgenographically several patterns have been described. In typical cases, two phases are seen in the evolution of the disease. In the early stages there are bilateral nodular processes which, with regression of the disease, are replaced by linear shadows. When emphysematous changes supervene, the diaphragms may become flattened. Cystic changes may be noted, giving the appearance of "honeycomb lung." Pneumothorax is not uncommon. Atypical cases also have been reported. Nadeau and associates (31) described a case which simulated metastatic carcinoma. The same writers also reported one case in which the initial densities were confined to the right lower lung, and after two months typical bilateral involvement occurred. Other writers have reported atypical cases (20, 23).

Nothing in the clinical or roentgenographic

picture specifically differentiates this disease from a large variety of pulmonary conditions which simulate it. For definitive diagnosis at the present time a lung biopsy is necessary.

In the treatment of this condition, antimicrobial drugs, corticosteroids, and radiation therapy have been used, with inconsistent results. Patients who have received no specific therapy have also shown improvement, but no patients, under any regimen, have shown complete roentgenographic clearing. Irradiation is considered to be contraindicated because of the possibility of intensifying the fibrosis which may be the natural end result of the disease (25, 31).

Summary

The present report describes a patient with pulmonary eosinophilic granuloma, followed for seven years, in whom spontaneous partial remission occurred. Pulmonary function studies at various stages of the disease are included.

Sumario

Granuloma Eosinófilo del Pulmón

Descríbese en esta comunicación a un enfermo de granuloma eosinófilo pulmonar, mantenido en observación siete años, en el cual sobrevino remisión parcial espontánea. Se incluyen estudios de la función pulmonar en varios períodos de la dolencia.

Resume

Granulome éosinophile du poumon

Le présent rapport décrit un malade ayant un granulome éosinophile pulmonaire, suivi pendant sept ans, chez lequel se produisit une rémission partielle spontanée. Sont inclus les études des fonctions pulmonaires à différents stades de la maladie.

Acknowledgments

The writers wish to express their gratitude to Dr. André Cournand, in whose laboratory the physiologic studies of July, 1955, were performed; and to Drs. Oscar Auerbach and Dicran Kassouny who assisted in the evaluation of pathologic studies.

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A SUMMARY OF THE THIRD CONFERENCE ON RESEARCH IN EMPHYSEMA[1,2]

AIR POLLUTION AND CHRONIC PULMONARY INSUFFICIENCY

Introduction

"Chronic pulmonary insufficiency" is a broad title tentatively chosen for a group of diseases which are generally labeled "emphysema" in the United States and "chronic bronchitis" in the United Kingdom. Some of its etiologies are "known" in a sense; for instance, sarcoidosis, mucoviscidosis, and certain pneumoconioses may cause either diffuse or focal emphysema. On the basis of pathologic and epidemiologic observations, various British observers believe that the disabling and frequently lethal phases of emphysema and cor pulmonale are simply late manifestations of a disease, *chronic bronchitis*, whose cause to them is known, i.e., *polluted air*, of both the community and individual (tobacco smoking) varieties, plus recurrent bronchial infection.

Patients with these diseases usually give long histories of recurrent respiratory *infections*; when sputum cultures are made, the predominant bacteria often prove to be *D. pneumoniae* and/or *H. influenzae*. Recurrent virus infections may play an important contributing role.

A genetic or acquired *defect* in one or more of the pulmonary *connective tissues* (elastic, reticular, collagenous, cartilagenous) may play a contributing—even a primary—role in these diseases. It is clear, for instance, that the trachea, bronchi, and bronchioles in advanced emphysema tend to be flabby and easily collapsible. Could this be a primary abiotrophy of, let us say, the tracheobronchial cartilage? It could, of course, be a *result* of mechanical trauma rather than the cause of airflow obstruction. Loss of lung elastic recoil is a classical finding in these diseases; but this, too, may be either primary or secondary, and it may be due either to a defect in the elastic or in some other tissue.

Alveoli have been shown by electron microscopy and other studies to be extremely delicate matrices of ground substance, elastic and collagen fibers, capillaries, and a mucoid material lining their surfaces. Physiologic studies indicate that alveolar tissue is potentially unstable: e.g., alveolar uniformity of shape and size is maintained in a somewhat precarious state of balance between collapse and overdistention by the combined forces of the connective tissue "snoods" and the *surface tension* of the lining material. In diffuse emphysema, it has been postulated that an excess amount of a detergent-like, surface tension–reducing substance is present.[3] This hypothesis thus places the primary process in the smallest air spaces rather than in the small or in the major bronchi.

A great many persons who have had chronic severe *asthma* for many years apparently end up with some degree of diffuse *irreversible* airway obstruction. Contrariwise, a significant proportion of patients with chronic pulmonary insufficiency have little or none of this so-called "bronchospastic" component.

Few observers still think that the loss of pulmonary *vasculature* which accompanies emphysema is a primary phenomenon, despite the very vital role that this change plays in the late clinical manifestations of the disease.

In summary, emphysema may prove to originate from one or more of the following: chronic recurrent irritation; chronic and/or recurrent infection; recurrent temporary or permanent, diffuse or patchy paralysis of the cilia; overproduction and increased viscosity

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[1] From The Webb Institute for Medical Research and the Department of Medicine, University of Colorado Medical Center, Denver 20, Colorado, edited by Roger S. Mitchell, with the help of Giles F. Filley and Morgan Berthrong.

[2] This conference was made possible by the Adams, Arapahoe, and Jefferson County Tuberculosis Association; American Thoracic Society; Boulder-Larimer Health and Tuberculosis Association; Colorado Tuberculosis Association; Crowley County Tuberculosis Association; Denver Tuberculosis Society; El Paso County Tuberculosis Association; Fremont County Tuberculosis and Health Association; Garfield County Tuberculosis and Health Association; Las Animas County Tuberculosis Association; Webb Institute for Medical Research; Weld County Tuberculosis and Health Association; Western Slope Tuberculosis and Health Association; and Grant No. H-5133, National Heart Institute, NIH, USPHS.

[3] Second Aspen Conference on Research in Emphysema, Amer Rev Resp Dis, 1960, *81*, 734.

of bronchial mucus; abnormally prolonged retention of various inert and/or chemically active atmospheric pollutants; an acquired or congenital defect or abiotrophy of one or more of the pulmonary connective tissues; injury from violent coughing. The widely varying clinical manifestations of this group of diseases seem best explained by postulating individual differences in the influence of these various factors of irritation, infection, trauma, and tissue defects.

The following summaries of papers were prepared by the participants themselves and follow in the order of their presentation at the conference.

SESSION I. GENERAL ASPECTS. THE PHYSICS AND CHEMISTRY OF THE AIR POLLUTANTS

Moderator: R. Prindle

THE AIR POLLUTION PROBLEM: KEYNOTE REMARKS[4]

RICHARD A. PRINDLE

There is no doubt that prevailing levels of urban air pollution constitute a problem. Many of the diversified effects of air pollution on man's environment are so visibly obvious that no one seriously questions that the problem exists. But when we begin to ask whether prevailing levels of urban air pollution present a health problem, the reasons why we suspect that they do are much less readily demonstrable.

There is abundant evidence pointing to an association between certain illnesses and causes of death and urban levels of pollution. What is more, experimental methods have shown that pollutants can affect physiologic variations from the homeostatic state, often leading to pathologic changes and occasionally resulting in death.

That there may indeed be a significant association between disease and air pollution is not really a new belief among physicians. Back in the 1930's, Dr. Haven Emerson, Professor of Public Health Administration at Columbia University, presented data which showed the association between the death rates for rheumatic carditis and the amounts of sunlight—according to latitude and smokiness—in several cities. He was, of course, far from the first to point out that, despite the lack of unequivocal evidence, there is a widely held opinion that "dark, smoky, foggy local atmospheres actually do cause a rise of local death rates. . . . When the air is dirty or smoky, cleanliness and ventilation are discouraged, light is reduced, plant life is damaged, spirits are depressed, and vitality is lowered."

It is now, however, at this relatively late date, that we are beginning to consider air pollution as a hazard to be controlled precisely because of its potential effects on health. Air pollution was not among those environmental hazards which were attacked in the nineteenth century when the "Era of Sanitary Awakening" began. For the most part, nineteenth century efforts to abate air pollution, as well as efforts in the first half of the twentieth century, gained public support because of the extreme soiling and nuisance caused by the problem. There are good reasons, as we look back, to explain why the public has waited until now to demand that air pollution problems be considered for abatement in the light of knowledge about health effects.

On the surface it appears strange that the evidence we already have pointing toward a very probable association between community air pollution and certain diseases has not provoked the modern public health administrator to immediate action. After all, a century ago mankind realized the benefits which could accrue from action vigorously taken and aimed at improving the general environment, despite meager evidence. Men such as John Snow, you will recall, acted on statistical evidence of association. Snow did not have proof, and he certainly did not perform studies which fulfilled Koch's postulates. What is more, in the nineteenth century a good deal less was understood about the complex and multiple causation of disease. We know better today than was known in the nineteenth century how difficult it is to focus on the specific cause of a specific malady, especially in the area of environmental health as it affects elderly patients already suffering from one chronic disease or a combination of them.

Since pragmatic efforts to improve public health by the elimination of community filth proved so effective in the nineteenth century, why do we not at this moment of history rid ourselves of the problem of air pollution which, in many urban areas, is no less a problem of filth than the old-fashioned open sewers? Well, I think we will rid ourselves of much of it. I am sufficiently optimistic to believe that the era in which we live may come to be known as the "Era of Sanitary Reawakening." However, there are extremely important differ-

[4] From the Air Pollution Medical Program, U. S. Public Health Service, Washington 25, D. C.

ences between the most widely recognized public health problems of the nineteenth century and the air pollution problem of today. The great difference lies in the nature of our goals and in the involvement of the problem with other aspects of our culture. Air pollution as an environmental problem today is an integral part, even though an undesirable one, of the industrialization, urbanization, and mechanization which characterize the technologic civilization which we highly desire.

Air pollution is so intimately involved with so many aspects of our culture that the health administrator finds himself with the difficult task of needing to take action in an area where health risks must somehow be balanced against real and imagined social benefits. In the nineteenth century this problem did not present itself in so formidable a fashion. The malaria mosquito and the typhoid bacillus, for example, against which health efforts in the early part of our century and the latter part of the last century were so successful, had no friends. Their presence did nobody good. When they typified our prime enemies, we were in a position to go all out to attempt to eliminate them absolutely. Today, however, to be in favor of vigorous action to eliminate environmental hazards absolutely is not so easy as it used to be. Society needs to know precisely how many and which types of air pollution sources must be curtailed or abated and to what degree. The health administrator must consider this demand, on the one hand, while keeping in mind the likelihood that no amount of air pollution is desirable insofar as the human respiratory system is concerned. It is not an easy task. It demands that we know much more than we do now, and that we find out much more as rapidly as we can.

This unique problem which the modern health administrator faces is so important that I feel compelled to illustrate it with a few examples.

If, for example, we should insist that death caused by highway vehicle accidents must be eliminated entirely, just as completely as we should like to eliminate malaria, what are we to say as to when the risk of death by high speed is equal to time saved? What is the time worth under what circumstance? How much time is worth how many deaths? How many deaths might occur from all other causes, if all vehicles were held stationary, which is the only way to eliminate absolutely the possibility of traffic deaths?

Are the risks of radiation damage, for example, comparable to the potential benefits of nuclear energy?

If I decide to drive from Washington to Boston, I know my risk of injury or death is greater than if I fly. I know that if I travel by train I would be even safer. I may decide, however, to drive, determining that time, cost, risk, convenience, pleasure, and other factors all properly added and subtracted indicate that in my opinion I should drive. I marshall the facts at hand and draw my conclusions before I act. This sort of decision, simple as it may sound, involves the same processes which must be followed in the solution of certain aspects of the air pollution problem. Questions such as these and many others must be asked and answered in the context of the cultural aspirations of the contemporary scene.

At present, we do not have enough facts to make completely rational decisions in the area of health and air pollution—not enough facts to know exactly how to act, what precisely to take action against, and to what definite purpose. Until we do, we can recommend only that the public take heed of the nineteenth century's progress in cleaning up filth through pragmatic experience.

The *purpose* of this conference is to encourage the research necessary to develop scientific facts which will enable our society to make decisions on a more rational basis. The *keynote* of this conference is that each of us is working on problems related in some degree to the health aspects of air pollution. The *plan* is to present what is known, some of the background, the chemistry and physics of the environmental aspects of the problem, the evidence that relates pulmonary disease to air pollution and, finally, the various facets of the many research activities involved. Starting with present knowledge, through physiology, down to studies at the cellular level, we may elucidate areas where basic research can help us.

I believe that through intense research efforts brought about by meetings such as this we will find a way to place in the public domain the basic health facts which must be known to assure the best possible political, social, and economic decisions—decisions which will be made largely by persons concerned only peripherally with the health professions.

Air pollution presents the kind of public health problem which challenges physicians greatly. But the challenge to people in other segments of society is equally great. It is everybody's problem. It is the problem of the manufacturer whose products or by-products contribute to air pollution, the city planner who influences our surroundings, the citizen who is adversely affected and also contributes his share to the problem.

Even though I have gone to great lengths to make clear my appreciation of the political, social, and economic ramifications of the air pollution problem, I want to make it equally clear that I believe that we can afford control. I quote from Dr. Porterfield, Deputy Surgeon General of the Public Health Service: "If there is enough money

to develop seven thousand new products each year, there is money enough in the richest nation in the world to control their by-product wastes. If there is money enough for motivational research to find out why people buy a product, there is money enough to make sure they are not injured by its existence."

We have come a long way since Emerson wrote: "It is the tragedy of today that man is so indifferent to the life of man. Yes, we surround the babe unborn with premonitory protection, deal wisely and gently with infancy and childhood, and then hurl the product of a reasonably healthy youth into a maelstrom of blind changes of dust, fumes and fatigue which wear down the stoutest body and cripple the most willing worker." We still have a way to go, however, before we can assume that Emerson's statement is entirely a part of past history but not of the present or the future.

AN EPIDEMIOLOGIC VIEW OF CHRONIC PULMONARY INSUFFICIENCY IN THE UNITED STATES[6]

EMANUEL LANDAU AND JEAN MORTON

During the past sixty years there has been a remarkable decline in mortality rates for all causes in the United States. Crude death rates for chronic bronchitis, asthma, and pulmonary emphysema also followed this downward trend, decreasing from 22.9 per 100,000 at the turn of the century to 8.7 per 100,000 for 1957. This contrasts greatly with the situation in England and Wales where chronic bronchitis is the third leading cause of death (1).

Why then our interest in what is now in the United States a relatively minor cause of death? It is the striking reversal of the above long-term decline in death rates for pulmonary emphysema. This is shown by comparison of the modest rate of 1.7 per 100,000 in 1900, which later fell to 0.2 per 100,000 in 1930 and 1940, followed by a steady slow rise from slightly less than the 1900 rate of 1.7 per 100,000 in 1950 to 3.4 per 100,000 in 1957—only seven years later. This increase has taken place uniformly among the sex and color groups of the population. Preliminary mortality tabulations for 1958 show a continuing increase in emphysema rates (2).

From analysis of vital statistics data, it has been determined that deaths from emphysema are not distributed uniformly throughout the United States, but are about twice as high in urban as in rural areas. From data provided by the National Office of Vital Statistics we have prepared maps showing the geographic distribution of emphysema deaths in the United States for 1950 and 1957. It is provocative indeed that in both years there was an overwhelming preponderance of deaths in the western one-third of the country with a striking increase (both relative and absolute) during that seven-year period. How can this be explained? Is this geographic distribution due to mobility, i.e., a shift westward of emphysematous persons to more beneficial climates? This could result in increased orientation of physicians in such areas to the presence of the emphysematous condition and acceptance of use of the term "emphysema" as a cause of death. Has this orientation and acceptability for death certification become so widespread a phenomenon that it has created a statistical artifact? Or does this represent an absolute and real increase in the disease?

Thus far we have been discussing pulmonary emphysema as the underlying cause of death. Let us also consider its importance as a contributory cause. The State of California conducted a study of multiple causes of death for 1955 from data obtained from the National Office of Vital Statistics in which it was found that emphysema was coded as the contributory cause of death more than twice as frequently as it was listed as the underlying cause (3). Consequently, this recognition of the possible importance of the striking increase in emphysema deaths in California led one of the authors (E. L.) to participate actively in a pilot study conducted by the California State Department of Health (4). This mortality follow-back study had as one of its objectives the determination of the diagnostic criteria used by the certifying physician when one of the chronic respiratory diseases was listed as the underlying and/or contributory cause of death on the death certificate. Another long-range goal was the assessment of environmental factors, including the possible contribution of air pollution to mortality from these chronic respiratory diseases. However, the pilot study itself was a methodologic study. It was designed to evaluate the relative efficiency of mail and personal interview techniques for the collection of data from physicians and next-of-kin. Use of the mail appeared to be equally effective at a substantially lower cost in terms of time and money than the interview technique.

However deficient the data on mortality from these particular chronic respiratory diseases may be, they are vastly superior to the data on morbidity which are virtually nonexistent except for a very few sources such as the National Health Survey. Very recently, the National Health Survey published a report on chronic respiratory conditions which gives such information as cases per 1,000 population, whether medically attended or not, and disability data including days of restricted

[6] From the Air Pollution Medical Program, U. S. Public Health Service, Washington 25, D. C.

activity, bed disability, and loss of time from work (5).

Valuable as the household survey type of approach may be for furnishing statistical estimates of the effects of a disease, it must of necessity run second-best to physical examination or measurement as an indicator of prevalence. One example of the latter is the current study which Dr. Hamill of our Air Pollution Medical Program has directed in which the goal has been to determine the prevalence and degree of chronic pulmonary insufficiency in two neighboring towns, four miles apart (Seward and New Florence, Pennsylvania), one with little air pollution and one with heavy exposure. In both towns, virtually the entire population thirty years of age and older was measured using the body plethysmograph, Wright peak flow meter, the polytesting Krogh spirometer.

To sum up, our concern is with the determination of the reasons for the increase in mortality of these selected chronic respiratory conditions in the United States. To that end, the follow-back type of study may be of considerable value in bringing about a greater measure of agreement on the diagnostic criteria used by physicians in certifying the cause of death. This type of approach may also enable us to compare in a more meaningful fashion mortality statistics for chronic respiratory conditions in the United States with those in Great Britain. Also, better estimates of chronic pulmonary insufficiency in the United States can be brought about by increased use of physical measurements in community studies.

Discussion: Many conferees believed that the true frequency of chronic bronchitis and emphysema in the United States is hidden under such other diagnoses as congestive heart failure, pneumonia and asthma, or may be incorrectly listed as a contributory rather than as a principal cause of death.

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RECENT EPIDEMIOLOGICAL EVIDENCE FROM THE UNITED KINGDOM ON THE RELATION OF ATMOSPHERIC POLLUTION AND COAL-DUST EXPOSURE TO CHRONIC NONSPECIFIC LUNG DISEASE*

J. C. GILSON

There are very striking differences in the crude male death rates from bronchitis in different countries in Europe. The rates per 100,000 are: England 80, Germany 16, Finland 2. Within Europe the rates are roughly related to the degree of industrialization, which suggests that atmospheric pollution and occupation may be important factors.

In England and Wales the death rates for both men and women are roughly twice as high in the connurbations as in the rural areas. In a special study of 80 county boroughs, Daly (1) concluded that about one-third of the difference in bronchitis death rates could be associated with differences in atmospheric pollution, and about one-third to the combined effect of such social factors as social class, over-crowding, population density, and educational standard. Reid (2) standardized for occupation by studying sickness absence records of postmen doing similar jobs throughout England. The wastage rate from bronchitis was very weakly correlated with population density, overcrowding, and climatic differences except for fog frequency. Absence rates from bronchitis, but not from other diseases, were highly correlated with a visibility index of atmospheric pollution, and the

* From the Pneumoconiosis Research Unit of the Medical Research Council, Llandough Hospital, near Penarth, Glamorgan, United Kingdom.

correlation, although present at all ages, increased strikingly with age.

The standardized male mortality ratios for bronchitis are raised in certain dusty occupations, e.g., coal-face workers (200 men), moulders (194 men), furnace-men (177 men), which suggest that dust may be a cause. This seems a less likely explanation when it is realized that the wives of the men in these occupations also have standardized mortality ratios which are high compared with those of the the wives of men in all occupations. These figures of the Ministry of Pensions and National Insurance show that the annual spells of sickness rates (per 100 workers) from bronchitis in mines (8.1) are about double that for all occupations (3.3), but this is apparently due to a high total sickness absence from all causes in the miners because, when the spells for bronchitis are expressed as a ratio of spells of sickness from all causes, the rate for the miners is the same as that for all occupations.

Thus, our national mortality and morbidity figures do not lend much support to the view that dust exposure, at least in miners, is a very important cause of bronchitis.

A much more detailed study of this problem in coal miners has been made over a number of years by my colleagues, Professor A. L. Cochrane and Dr. I. T. T. Higgins (3-6), in age-stratified geographically defined randomly selected population samples. Using a standardized questionnaire of respiratory symptoms and smoking habits, a chest roentgenogram, and the forced expiratory volume over 0.75 second, they have been able to compare miners and ex-miners against those without mining experience (nonminers) living in the same area.

They find that the prevalence of bronchitis (defined as persistent sputum for at least three months in the year and at least one chest illness in the last three years) and the ventilatory capacity are unrelated to the severity of dust exposure as measured by the roentgenographic category of simple pneumoconiosis. Miners and ex-miners have, however, more bronchitis and a lower ventilatory capacity than the nonminers, but the difference between the nonminers and miners and ex-miners varies strikingly in different areas. The biggest difference is observed in South Wales. The roentgenographic category of simple pneumoconiosis is well related to the total dust content of the lung; those with category 3 (I.L.O. Classification 1950) have five times as much dust in the lungs as those with category O (7). There is evidence to suggest that, for all ages, those with a very short exposure (less than one year) on the coal-getting shift, which is the dustiest job, have fewer symptoms, less bronchitis, and a higher ventilatory capacity than those who have spent longer on the coal-getting shift; but that, for periods longer than one year, the symptoms, bronchitis and ventilatory capacity, are not closely related to the period of exposure. These studies have also emphasized the importance of cigarette smoking as a cause of bronchitis and impairment of ventilatory capacity. The difference between the nonsmokers and smokers is as large or larger than that between the miners and nonminers.

To conclude: Epidemiologic studies in the United Kingdom have not identified a single major factor in the genesis of bronchitis and ventilatory impairment. Exposure to coal dust is probably less important than is often supposed. Personal pollution by cigarette smoke is probably more important, while general atmospheric pollution is likely to be a factor. However, the type of pollutant is at present uncertain. Well-controlled international epidemiologic studies might be of considerable help in establishing the relative importance of some of the factors, such as smoking, because there are countries with a low bronchitis death rate and a high cigarette consumption.

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MINOR COMPONENTS OF THE AIR AND THEIR BIOLOGICAL SIGNIFICANCE[1]

A. J. Haagen-Smit

Mass toxic effects as occurred in London and in the Meuse Valley, and especially eye-irritating clouds over Los Angeles, have been most effective in drawing the attention of the public and the medical profession to the quality of the air we breathe.

Chemical analysis of city air shows the presence of a large number of components other than nitrogen, oxygen, water, carbon dioxide, and noble gases. The concentration of these minor components is highly variable and is usually in the range of one part per million or less. Notwithstanding their occurrence in such low concentrations, they can have a profound effect, acute or long-range, on the behavior of organisms, plants as well as animals. The nature of these minor components varies with the activities of the community, and a knowledge of the emissions gives a first approximation of the composition of the polluted air. Direct analysis of the air finds many of these substances present but, in making up a balance of the amounts emitted and those actually found in the air, we often find a large discrepancy. Not only have considerable quantities of the emissions disappeared, but whole series of new compounds are detected for which no direct source of emission can be found.

The classical example of this phenomenon is furnished by the conditions prevailing during Los Angeles' type of air pollution. In this area nearly 2,000 tons of hydrocarbons, mainly of petroleum origin, are emitted to the atmosphere daily. Spectrographic analysis of Los Angeles air finds only a part of the originally emitted material, while the rest is present in oxidized form as organic acids, aldehydes, and ketones. In this process new pollutants are formed, such as ozone, which is present in the Los Angeles atmosphere in concentrations as high as 0.6 parts per million. The total tonnage present in the basin during smoggy days is calculated to be of the order of several hundred tons. Actually, there are no significant direct ozone sources at ground level, and the presence of an inversion layer effectively prevents diffusion of ozone from the upper atmosphere. We know now that ozone is formed in a photochemical oxidation process of hydrocarbons in the presence of oxides of nitrogen. The oxides of nitrogen as well as those of sulfur, mainly released as nitric oxide and sulfur dioxide, are also subject to atmospheric influences. These are thereby converted to higher oxidation stages and through subsequent reactions with alkaline material are found as nitrates and sulfates.

These investigations have shown that the composition of the polluted atmosphere is continuously changing, and we should regard the atmosphere as a dynamic system which gradually approaches the state of greatest stability, which, with sunlight and oxygen abundantly present, would be carbon dioxide, water, nitrates, and sulfates.

Unfortunately, at the low concentrations of one part per million and less, even so-called fast reactions may take considerable time for completion. The reactions leading to these final products pass through many intermediates and the Los Angeles population is painfully aware of being caught in this process of atmospheric assimilation of the new constituents. Modern methods of analysis, gas chromatography and spectroscopic analysis, have given us the tools to unravel a substantial part of this mixture. In this way many substances have been discovered to be present in the air which await testing of their physiologic or toxic effects on animals and men. Testing of the large number of identified molecular species, useful and necessary as it is, may not give a true picture of conditions actually prevailing in our atmosphere.

In the series of reactions leading to the Los Angeles type of smog, many short-lived intermediates are formed. In addition, primary gaseous pollutants are transformed into less volatile reaction products forming aerosols. These aerosols together with those originally emitted as smokes and fumes pose difficult problems in analysis and especially in the evaluation of physiologic and toxic effects of the combined gaseous and particulate matter.

A typical case of the gradual change in composition and toxic effect of air pollution clouds has been noticed on plants subjected to the oxidant type or the Los Angeles type of smog. In such atmospheres the plant pathologist recognizes two types of damage, one caused by products of the oxidation of organics and the other by ozone, a by-product of this oxidation.

Through a difference in oxidation rate of the hydrocarbons and differences in stability of the plant-damaging factors, we find oxidant damage limited to the neighborhood of the source while ozone damage appears at greater distance. Such observations on variations in plant damage pattern through differences in reaction rates of pollutants are of equal importance in the study of physiologic and toxic action on animals.

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THE PHYSICOCHEMICAL BEHAVIOR OF SUBMICRON AEROSOLS*

ALEXANDER GOETZ

The vast multiplicity of nature's energy conversion processes results in perpetual interactions between the surface of our planet and the lower strata of the atmosphere to which man contributes at a rapidly increasing rate.

These processes result not only in gradual changes of the gaseous components of the atmosphere but also in the suspension of solid and liquid matter in particulate form. The duration of their suspended state can vary between minutes and possibly centuries, depending entirely upon the physical and chemical nature of the particles. Fallout by gravity ceases to be a significant cause of removal for particles below about 3 micron in diameter. Here begins the size range of the aerosols; here also begins the gradual failure of the protective barriers against particle penetration into our respiratory system.

The constitution of such aerosols embraces solids, liquids, or even temporary statistical molecular agglomerates so small in size and mass that gravity does not affect them any more significantly. The ever present atmospheric vertical convection would keep them air-suspended for indefinite periods were it not for their occasional but only partial precipitation by rain. It follows thus that air, free of such suspended matter, is extremely rare.

Contrary to the fairly well-defined upper size limit of aerosols, their lower limit is quite vague, for it involves the definition of the borderline between a molecule (or a macromolecule) in the free (gaseous) state, and a particle. The term "particle" is probably applicable when the surface of a molecular complex is sufficiently permanent to result in a reversible interaction with the suspending free gas molecules for a long period compared with the interval between the collisions by the former.

However, a lower size limit is predictable for all substances of appreciable vapor pressure on thermodynamic grounds: the Kelvin relation requires a rapid volatility increase of a particle with the decrease of its diameter, particularly for material of high molecular weight and small surface tension, i.e., many organic compounds. This results in a rapidly decreasing stability of the very smallest sizes (10^{-7} cm.) and condensation on particles of lesser surface curvature.

The most common lower size limitation is coalescence, whereby at sufficient concentration the smallest, most mobile units agglomerate by mutual collision into larger, more inert particles, thereby decreasing rapidly in number and probability of further collision. This process results in a virtually permanent optimal size and concentration, for only those which specifically happen to grow beyond a critical size will gradually precipitate.

Such quasi-stable concentrations result in nature in 100 to 1,000 particles per cubic centimeter with a size range from about 0.2 to 0.7 micron. Local conditions can increase this concentration temporarily by ten to one hundred times.

The chemical constitution can also be a major factor determining the stability of the smaller sizes —as well as the lifetime of an entire aerosol, particularly for organic substances—since as particulates they are subject to efficient photochemical conversion processes because of the drastically increased accessibility of the particle constituents to interaction with light quanta.

Because such aerosols are insignificant with respect to mass, their chemical or spectroscopic identification is quite evasive. For example, at a (normal) concentration of 100 γ per cu. m., the particles represent but one ten-millionth (10^{-7}) of the mass of the supporting gas molecules, although this minute fraction constitutes more than 3½ million particles of 0.3 micron diameter in the volume of each breath.

Of definite physiologic interest is the capacity of such particulates to act potentially as accumulating centers (nuclei) for condensable, toxic, or irritant gaseous traces in the atmosphere. Synergistic intensification of such physiologically neutral particulates can be expected if the toxic condensate is but loosely associated with their surface so that its transfer onto the tissue results after the deposition of the particle thereupon. The intensification is apparently due to the large increase of topical irritant concentration. The reverse effect, i.e., an attenuation, is observed when the affinity of the condensate to the particle substance is sufficiently large to prevent transfer. Both effects have been observed in animal tests (LeBelle, Amdur and associates).

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These synergistic phenomena are thus characterized by the following:

1. They depend on the nature of both the particle and irritant gas phase.
2. They depend on the mean particle size, so that the larger and the smallest sizes are less effective per unit mass; the former is due to the smaller total surface, the latter because of the Kelvin effect (a.a.). The most effective size should vary with the material involved.
3. They are noticeable only at low gas concentrations, and then increase with the concentration of the aerosol.
4. They should be dependent largely on temperature and relative humidity, and their increase dependent on the decrease of one or both qualities.

The currently still scarce experimental data indicate that these relations exist, provided that no chemical or catalytic interaction between particle and gas originates a new substance of larger or lesser toxicity.

Another effect caused by this associative capacity, though restricted to hygroscopic aerosols, is their increase in size and mass, caused by the accumulation of water vapor when introduced into the high relative humidity of the respiratory system. This renders the particles less mobile and thus more interceptible, and represents a potential mechanism which makes their exhalation less likely. A typical example is a submicron aerosol of propylene glycol where the induction into an artificial model of a respiratory channel reduces the mobility eight- to tenfold and increases the particle mass (by vapor absorption) 500 to 1,000 fold (Goetz).

In judging the role of aerosols, it appears indicated to determine their kinetic (et cetera) properties under the conditions prevailing within the respiratory system; furthermore, to compare the physiologic reaction remaining after the removal of the *intact* aerosol particles with the effects prevailing in their presence. This requires a method of subtracting the aerosol from the gas phase under controlled conditions to assure no interference with the status of the particle and its potential condensates.

The separation by the aerosol spectrometer in a high contrifugal field out of a nonturbulent continuous air stream involves no appreciable shear, or changes of temperature, or pressure. Systematic studies of natural and artificial aerosol systems with this method during recent years have shown that it can render the large variety of such problems accessible to evaluation and thereby to a much-needed basic understanding.

THE ORIGINS AND IDENTITIES OF IRRITANTS IN POLLUTED ATMOSPHERES[9]

LESLIE A. CHAMBERS

The preceding papers by Drs. Haagen-Smit and Goetz have indicated some of the classes of particles which are known to occur in the atmosphere, and have shown that many of the pollutants exist in highly unstable states, implying that their physiologic effectiveness must vary from time to time. Thus, we have a partial description of the contents of the air in which man must live.

It is a logical next step to consider the alterations of physiologic and biochemical functions which may be induced by tissue contact with these highly variable substances. At present, it is necessary to consider exposure of pulmonary surfaces to *effective* dosages of the pollutants. In most cases, we know the kinds of qualitative responses to be expected, but in very few instances have we any knowledge of the concentration-time relationships necessary to cause a detectable reaction.

Materials brought into contact with respiratory tissues by the breathing process may be grouped into at least eight categories with relation to the types of physiologic response they are capable of eliciting.

1. Relatively inert particles which are abundant in most atmospheres and which are generally represented by the nonspecific terms "dust," "fly ash," "particulate matter," et cetera, are capable of stimulating a variety of reflex neural mechanisms such as coughing and sneezing and, when present under appropriate conditions and in sufficient quantity, seem able to elicit benign isolation processes such as fibrotic encapsulation.
2. From the enormous variety of chemical species already identified as pollutants of the air, some and perhaps a great many are able to affect specific cellular biochemical processes, resulting in the suppression of normal expulsive responses. This has been demonstrated in relation to ciliary activity of epithelial cells. These and other interferences with normal metabolic functions can be supposed to act variously to promote hypo- or hypersecretion, cellular necrosis, and other abnormal responses, including neoplastic growth.
3. Strong acids, alkalis, certain metallic compounds, and other substances, are known to be capable of causing localized direct structural changes of a nonspecific character. In other words, they are corrosive.

[9] From the Allan Hancock Foundation, University of Southern California, Los Angeles, California.

4. Allergenic substances, in addition to pollens and related materials of natural origin, must be assumed to be present in the atmosphere. The extent of occurrence of substances containing potentially allergenic chemical groups requires much fuller examination. In a study of alpha-amino nitrogen distribution in the atmosphere of American cities, it was found that a surprisingly high concentration of this biologic indicator was present, much higher than could be accounted for as bacteria and pollen. Furthermore, the highest concentrations were found to be present in heavily industrialized, metropolitan areas rather than in rural regions. It would be interesting to learn how much of the discomfort associated with air pollutants can be related to allergens.

5. In recent years, at least some of the pollutants contained in the atmosphere are, or have been, radioactive. Generally, it is the habit to consider the effective dosage of such radioactivity in terms of a volume of air. However, the radioactivity is not uniformly distributed, but occurs in association with discrete particles. Bits of matter, exhibiting many thousands of disintegrations per minute, have been isolated. While the probability of impingement on a pulmonary surface is relatively small, one cannot help speculating on the dosage of radiation administered to the volume of a few cells by the retention of such a particle for any significant time. Unquestionably, the amount of radiation locally would be well above toleration limit. Whether or not an isolated event of this character would be sufficient to induce extensive pathologic processes apparently is unknown. Certainly, it may be assumed that any considerable number of minute radiation injuries increases the probability of mutative responses presumed to underlie the induction of malignancy.

6. The atmosphere in any inhabited area carries some visible pathogenic microorganisms. The disease processes caused by them are presumably not within the scope of the present conference.

7. The atmosphere may contain measurable amounts of substances such as lead and beryllium which exert no direct effect upon pulmonary systems, but are cumulative and may be either organ-specific or systemic poisons.

8. On the other hand, it is well known that some other materials such as hydrogen sulfide and carbon monoxide are representative of compounds which do not accumulate and do not have any direct influence on pulmonary tissues, but may be organ-specific or systemic poisons. One may illustrate the basis for this classification by references to either the episode involving hydrogen sulfide at Poza Rica, Mexico, or the application of hydrogen cyanide in gas chambers.

Of these eight classes of materials, the first six produce effects directly on pulmonary structures. The cumulative or acute poisons of groups 7 and 8 are probably of little direct significance in the etiology of abnormalities of the respiratory system.

The effective transportation of air-borne substances to susceptible areas is influenced by the size of the particle and other physical properties such as electrical charge and hygroscopicity, and by the respiratory morphology and breathing habits of individuals.

Available analyses of polluted atmospheres are very limited. Information on reaction processes associated with contaminated air leads to the conclusion that representatives of all eight categories of physiologic affectors are inhaled by inhabitants of most cities. The present lack of information on minimal effective dosages makes it impossible to draw conclusions as to the significance of amounts currently being inhaled.

The combined "dosage" effectiveness incurred by contact with two or more pollutants, administered simultaneously or in some time sequence, is at present a vaguely conceived area of probable importance. Gradually, the notion is growing that the net pathologic responses can be understood only in terms of multiple and cumulative insults.

The origins of many species of pollutants can be determined; but, in many other cases, the circumstances giving rise to their formation in the atmosphere are complex and very poorly defined. Even when identified at a point in time and space, the duration of their existence is so dependent on adjacent components of the pollutional complex that the concentrations actually able to reach biologic receptors are indeterminate without reasonable knowledge of the entire system.

The relationship of air pollution to emphysema is largely speculative in terms of direct evidence, but logical attractiveness of air-borne irritants as factors in the pathogenic process suggests the need for examination of the hypothesis.

CONSIDERATIONS OF PARTICLE PHYSICS IN CAUSATION OF PNEUMOCONIOSES[10]

THEODORE F. HATCH

The physical properties of dust particles are of primary concern in the full delineation of etiologies of dust diseases, beginning with consideration of the source material and dust-producing process and continuing with an understanding of the behavior of dust in the atmosphere and extending into the respiratory system. Even after deposition of the dust in the lungs, physical properties

[10] From the Department of Industrial Health Engineering, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

continue to play a role in determining the kind and magnitude of dust disease which results. The physical properties have so much influence, in fact, that, for the same kind (chemical) of material and for equal conditions of exposure in other respects, differences with respect to one or another physical characteristic or property can cause wide variation in consequence, ranging all the way from serious risk of disease to literal freedom from hazard. A full understanding of the ways in which physical properties of dust modify etiologies and relative risks, therefore, is essential to the proper use of "occupational history" as an aid in diagnosing particular cases of suspected dust disease.

Characteristics of source material and of dust-producing process: The dust produced from pulverizing complex rocks frequently differs in its mineralogic makeup from the parent material, owing to unequal degrees of shattering of different components in the rock. Thus, the free silica content, for example, of air-borne dust may be quite different from that of the original rock and, even within the dust, the percentage of SiO_2 can vary greatly with particle size. The air-borne dust settles under gravity at velocities proportional to size and, in consequence, the mineralogic composition of the dust can vary with time or with distance from source.

Particle size of dusts varies with method of dust production and, of greater interest, the degree of agglomeration of particles varies widely from one process to another. These differences in size characteristics, together with differences in mineralogic composition with size, can result in some remarkable changes in risk of dust exposures quite unrelated to the nature of the original material from which the dust was produced, thus complicating the essential etiologic relationships between dust exposure and health risk.

Behavior of dust in the respiratory system: Physical properties of the dust continue to operate as the dust is inhaled into the respiratory system. The airways and pulmonary spaces operate as a dust-collecting system, the operating characteristics of which are determined, as in any other dust collector, by physicodynamic considerations. Particles of different sizes have different probabilities of penetrating to, and being deposited at, sites of various depths within the respiratory system and, for a given size, the probabilities vary with the breathing volume and frequency distribution of new air in the lungs, et cetera. For typical mineral dusts and normal respiration, there is maximal deposition in the pulmonary spaces of 1 to 2 μ particles, of the order of 50 to 60 per cent. Larger particles are trapped along the upper respiratory tract, and particles smaller than 1 to 2 μ (down to about 0.5 μ), although they may penetrate to the pulmonary spaces, are not caught, owing to low settling velocities and insufficient time of stay of air in the lungs. The percentage deposition in the pulmonary spaces is limited, too, by the fraction of each tidal volume which reaches the lungs. Below 0.5 μ, particles have negligible settling velocities, but their Brownian motion becomes increasingly active, and such submicroscopic particles are deposited by the diffusion process. Thus, as size decreases below 0.5 μ, lung deposition increases and, below 0.05 μ, it becomes as high as for 1 to 2 μ particles, the limit again being fixed by the fraction of tidal air which reaches the lung depths. For these ultrafine particles, too, there is greater probability for deposition in the alveoli than for the 1 to 2 μ particles, which are more likely to be deposited along the respiratory bronchioles and alveolar ducts. The reason is that the coarser particles penetrate only to the depth of active mechanical ventilation whereas the submicroscopic particles, like gas molecules, may diffuse out of the zone of active air replacement into the relatively static alveolar gas. These differences in potential sites of deposition (alveoli vs. vestibular channels of the pulmonary lobules) can have great significance in respect to potential physiologic and pathologic effects.

Fate of dust in the lungs: Dust deposited along the ciliated surfaces of the normal upper respiratory tract is rather quickly removed—within a matter of hours, at most. Depending on chemical makeup, these coarser particles can produce local irritation or they might produce toxic effects after being swallowed, but they do not cause deep-lung damage. Particles deposited in the lungs may also be removed by way of the upper respiratory escalator, but at a much slower rate. The mechanism whereby particles are conveyed to the ciliated channels for removal is not well understood. Phagocytosis plays an important part and there is good evidence that the removal rate goes down with size and varies, as well, with the physicochemical nature of the particles. Contrary to earlier views, the current idea is that phagocytosis acts altogether as a protective mechanism and that movement from the alveoli into lung tissue occurs without prior engulfment of particles by phagocytes. Rates of lung clearance have varied in animal studies from half-life values of a few days to many months. In silicotic lungs, only a minute fraction of residual dust is found, compared with the estimated quantity taken in over the years of exposure. This suggests that the ultimate disease is associated with only a small and, presumably, highly selected fraction of the dust originally inhaled.

It is clear from this brief review that there are many complicating physical factors which enter

into the etiology of dust diseases, all of which can have significant influences on the relationships between level of dust exposure and magnitude of *effective* dose of dust to the critical site within the respiratory system. The relationships may be relatively simple in the case of "classical" silicosis, but, unquestionably, they become increasingly complicated as we look at the nonspecific pneumoconioses and consider the possible role of dust exposures in the development of "chronic pulmonary insufficiency."

ON THE OCCURRENCE OF FREE RADICALS IN AIR POLLUTANTS[11, 12]

M. S. Blois

It has been predicted that the chemical and photochemical reactions of volatile wastes and primary atmospheric pollutants may involve free radicals (1) as reaction intermediates. The lifetime of low molecular weight, nonaromatic, free radicals is ordinarily extremely short, although recent studies on free radicals in solution have revealed mechanisms of stabilization which may result in the conversion of these radicals to a more stable form (2). A question remains as to whether free radicals in the atmosphere may undergo further reactions to achieve a similar stabilization.

Apart from these considerations, there has been much speculation that free radicals are biologically toxic, this being presumably based upon their characteristic chemical reactivity. More specifically, they have been incriminated in carcinogenesis, in the biologic effects of ionizing radiation, and in the process of aging. We will not concern ourselves here with the arguments pro and con respecting this hypothesis or the details of these hypothetical mechanisms. However, to evaluate any postulated etiologic role they may have, it is first necessary to establish their presence in a human environmental situation. Thus, if free radicals in air pollution are to be invoked in the etiology of chronic pulmonary disease, they must not only be shown to occur in polluted atmospheres but, in addition, to have appropriate lifetimes, reactivities, and concentrations.

Recalling that, by definition, a free radical is a molecular fragment with an odd number of electrons, two general methods of study suggest themselves. First are those chemical reactions which indicate either a change in oxidation state or a response to the introduction of free radicals by undergoing polymerization or crosslinking, both types of behavior resulting from the donation or acceptance of one electron to or from the initiating radical. The first type of chemical reaction is exemplified by the oxidation-reduction dyestuffs (indicators) or by the stable free radical, α,α-diphenyl-β-picryl hydrazyl (DPPH) (3). The second scheme (4) has been used by radiobiologists for radical counting in solution and may be useful for free radicals in the gas phase as well (5).

A more general approach to the study of free radicals is the use of electron paramagnetic resonance (e.p.r.) (6, 7) which is a purely physical phenomenon depending upon the free radical having an odd, magnetically uncompensated electron. It had been reported previously (2) that cigarette smoke condensed out at liquid nitrogen temperature showed the presence of free radicals by e.p.r., and that the rate at which the free radical concentration fell as the sample was thawed suggested that there were "stable" and "non-stable" classes of free radical present.

We have performed preliminary experiments in which cigarette smoke was bubbled through a tall column of DPPH in a standardized, cyclic pattern, and the resulting decolorization was taken as a measure of the free radical concentration in the smoke. The DPPH decolorization was studied as a function of length of the cigarette, and was observed over a period of time after exposure. It was noted that a certain degree of decolorization occurred immediately upon exposure to the smoke, and that bleaching of the DPPH continued by as much as another order of magnitude over the ensuing twenty-four hours. These observations are consistent with the view that perhaps one-tenth to one-third of the radicals detected were highly reactive alkyl and low molecular weight species, whereas the remainder were less reactive species and probably included the aromatic hydrocarbon radicals.

Although the DPPH method is widely used for radical counting, the interpretation of results is not without ambiguity, and the e.p.r. technique is by far the more direct. For this purpose, particulate atmospheric pollutants collected on a glass fiber mat by the Los Angeles County Air Pollution Control District were examined. This material showed an asymmetric e.p.r. signal with a g-value of about 2.1 and a line width of approximately 500 gauss. When the sample was put into aqueous suspension and then redried, the original broad line was essentially unchanged and a new, narrow line was present with a g-value of 2.0033. Estimates of the number of unpaired electrons in this material were made by using the electron spin resonance (e.s.r.) absorption intensity, the e.s.r. line width,

[11] From the Biophysics Laboratory, Stanford University School of Medicine, Stanford, California.

[12] These studies have been supported by a grant from the National Institutes of Health, U. S. Public Health Service (A-2304).

and by the DPPH method. These results were respectively 20×10^{20}, 20×10^{21}, and 5×10^{19}, the units being unpaired electrons per gram. The sample was repeatedly examined over a period of about two months after collection with no apparent loss of activity.

For purpose of comparison, smokes from burning cigarettes and from paper were collected on cotton filters. Examination then revealed e.p.r. signals with g-values of 2.0031 and 2.0027 and line widths of 8 and 4.5 gauss, respectively. These very preliminary observations do not permit generalization. It does seem certain, however, that paramagnetic materials are indeed present in particulate atmospheric pollutants, and from their g-values it seems probable that at least a part of this signal is due to stabilized free radicals. It is significant that this apparent free radical activity appears stable in air for extended periods, yet reactive when placed in an aqueous system, this combination being especially meaningful in the context of this conference.

The next step must be to extend these results, to attempt the identification of the species involved, and to account for the curious combination of apparent stability and potential reactivity. It would appear that many direct molecular biologic observations are feasible which would illuminate the etiologic problem, in addition to surveys of free radicals in air pollutants in connection with the epidemiology of chronic pulmonary disease.

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SESSION II. THE PATHOLOGIC EFFECTS OF THE AIR POLLUTANTS ON LUNG TISSUES

Moderator: J. Wyatt

THE INTRAPULMONARY MOVEMENT OF AEROSOLS[18]

Norton Nelson

In the course of studies on the physiologic handling of aerosols in the respiratory system (Altshuler, B., *et al.*, AMA Arch Industr Health, 1957, *15*, 293) it became apparent that submicron particles exhibited rather striking stability and passivity within the respiratory air spaces. This behavior plays a significant part in the movement of particulates within the air spaces of the lung and the pattern of their deposition. It was predicted many years ago by Findeisen that, as a consequence of the decreasing influence of gravity and inertial forces with decreasing particle size and the simultaneously increasing effect of molecular bombardment or Brownian movement on particle transport, particle deposition in the respiratory tract would show minimal deposition efficiency at an intermediate size. This prediction was confirmed in this work; with particles of unit density this minimum was found with particles approximately 0.5 micron in diameter. With mouth breathing the total deposition at this size is about 10 per cent and increases with both larger and smaller particle sizes. This small deposition and large return of inhaled particles of this size illustrates the stability of such particles suspended in respiratory air. This stability is further shown by the fact that individual particles persist in the respiratory tract over a number of respirations, escaping both deposition and exhalation. This behavior is vividly shown when doing a washout curve breathing clean air after inhalation of aerosol: the aerosol concentration in the end-expiratory samples decreases in smooth and measurable manner over a number of respirations.

These studies have been carried out by a group in our laboratory, including Drs. Altshuler, Palmes, and Yarmus; they were made practicable by two factors: one, the availability of particles produced by the LaMer aerosol generator substantially homogeneous in diameter; and, two, an

[18] From the Institute of Industrial Medicine, New York University—Bellevue Medical Center, New York, New York.

instrument employing the Tyndallometric principle capable of measuring instantaneous aerosol concentration in expired air during the respiratory cycle.

The stability of submicron particles arises from the limited and minor forces acting to move them from the airstream. A particle of 0.5 micron in diameter has no independent motion, being subject to transport, as noted above, by Brownian motion and the residual, inertial, and gravitational forces. Because of this passivity, particles will tend to stay rather closely within a geometric air unit; that is to say, they will tend to follow air currents and will show only a limited drift by molecular bombardment. Thus, during the steady-state inhalation or respiration of air containing fine particles, these particles will follow the tidal volume up to the virtual interface separating tidal volume and functional residual air. Gas molecules will move rapidly across this interface due to their high kinetic velocity. Submicron particles on the other hand will move across it very sluggishly. When this interface exists within the alveolar portal, the mean free path of carbon dioxide and oxygen is more than adequate to transport molecules between the interface and the respiratory surfaces. On the other hand, the limited movement of particles does not permit this rapid transfer. Accordingly, one would not expect to find a sharp concentration gradient for gases at this interface between tidal and residual air, but one should find a sharp gradient in aerosol concentration. Recent work by Dr. Altshuler shows the presence of this gradient very vividly. This can be demonstrated by the inhalation of a fixed volume of aerosol-laden air followed by a forced exhalation which serves to move this interface past the recording Tyndallometer. The interface shows a sharp decrease in concentration, and, in curves normalized for the amount inhaled, there is a striking similarity over a large range of tidal volumes.

If there is this sharp concentration boundary—and it now appears that such is present—it must necessarily play some role in the pattern of particle deposition within the end respiratory units. This might well lead to rather sharp gradients in particle deposition along the alveolar duct and respiratory bronchiole and also in the accumulation of phagocytes for transport of deposited particles.

These findings have no immediately apparent application to air pollution. However, particle behavior at this critical region of the respiratory system is of obvious importance for the action of particulate toxins either locally or systemically and will require further examination.

MEASUREMENT OF HUMAN BRONCHIAL GLANDS IN CHRONIC BRONCHITIS: EXPERIMENTAL STUDY OF THE RESPONSE OF THE BRONCHIAL TREE TO IRRITATION; TYPES OF EMPHYSEMA IN CHRONIC BRONCHITIS[14]

LYNNE M. REID

The significant and constant clinical feature of chronic bronchitis is the production of sputum; less constant features are infection and breathlessness. For purposes of epidemiologic studies the category has sometimes been taken to embrace all three, but a truer picture of the incidence of the disease is gained by including only the fundamental feature, sputum production. Such studies demonstrate a definite relationship between the degree of atmospheric pollution and the frequency and severity of chronic bronchitis. Before the nature and extent of the effect of atmospheric pollution can be analyzed, however, it is necessary to see whether the clinical features referred to are reflected in structural changes which are clearly distinguishable from the normal. To this end, measurements have been made of the size of the glands in normal lungs and compared with those of patients who produced sputum persistently for no less than five years (1); measurements of gland and acinar diameters and wall thickness have been taken and have shown that the change is so marked that bronchitics can thereby be distinguished from normal subjects. Furthermore, they have shown that emphysema can exist without gland hypertrophy, i.e., evidence of chronic bronchitis, in the large bronchi. Apart from gland hypertrophy, there is a corresponding increase in the number of goblet cells in peripheral bronchioles, i.e., the production of mucus is also increased in the lung periphery (2).

Infection is probably the most important single cause of the peripheral damage which occurs over the years in patients with chronic bronchitis. It is, in general terms, of two kinds: fibrosis or scarring, and emphysema, by which last is meant abnormally large air spaces distal to the terminal bronchiole. Both kinds of damage give rise to "clinical" emphysema, but only the second is emphysema in the "pathologic" sense. Two forms of the latter may be seen: one is the ulceration of lung tissue, the "destruction" type, often explained by the micro-abscesses seen scattered irregularly in the lung periphery in cases of chronic bronchitis (3);

[14] From the Institute of Diseases of the Chest, Brompton, London, England.

and the other is the "distention" type, in which the lung is architecturally intact (4).

Commonly, the natural history of chronic bronchitis comprises intermittent bouts of infection with the ultimate development of breathlessness. The role of bacteria in the bronchial tree may take on a number of forms, acting as a surface irritant or attracting polymorphonuclears through the epithelium or invading and ulcerating the epithelium (5).

The question which presents is how mucous secretion is related to infection. Do atmospheric irritants damage the bronchial wall directly or only by creating a condition in which infecting organisms can take hold? To investigate this problem, experimental animals have been exposed to sulfur dioxide and it has been found possible to induce an increase in goblet cells while the lungs are still sterile, suggesting that direct irritation causes an increase in mucus-secreting cells while the trachea, bronchi, and lungs are free from infection (5). In animals it has been shown that surface irritation causes glandular secretion by a nervous reflex (6) and it could be that in this fashion the glands suffer a work hypertrophy. The working hypothesis is suggested that hypertrophy of mucus-secreting structures is the result of different irritants, which makes infection more probable.

The abnormal production of mucus is characteristic of chronic bronchitis at all stages of its natural history and may be its only manifestation. All too often, however, it is complicated by damage to the respiratory part of the lung, leading to permanent disability and perhaps death.

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During discussion of her paper Dr. Reid made the following additional comments.

1. The structural complexity of the lung is such that it may be meaningless to talk in general terms of the contribution that atmospheric pollutants make to lung damage without analyzing the nature of any damage, the site at which it occurs, and the mechanism by which it has developed. For example, particulate matter may act on the bronchial wall to cause hypersecretion of mucus and perhaps hypertrophy of the gland layer, while, when it enters alveoli, it becomes stored in macrophages with resulting morphologic modification of the alveolar wall. Furthermore, the changes in the bronchi associated with infection may give rise to secondary changes in the alveolar or respiratory part of the lung and it may be that it is this indirect type of damage which has the more serious functional implication.

2. In the experimental production of emphysema, a variety of mechanisms may produce appearances similar to those seen in the human lung, answering a simple pathologic criterion of emphysema, i.e., dilated airspaces beyond the terminal bronchiole. From the standpoints of functional derangement and reversibility, however, there may be no resemblance to the clinical and pathologic pictures of the disease in man; furthermore, the mechanisms used to produce emphysema experimentally may find no counterpart in those operating in the patient.

3. In the United States it has caused surprise that the mortality figures for the United Kingdom put chronic bronchitis so high on the list of fatal diseases. It is, of course, the complications which are fatal. Etymologically, the term refers properly to changes in bronchi but, although in the United Kingdom when disability is great the diagnosis "emphysema" is usually added, the term "chronic bronchitis" sometimes embraces the whole range of conditions from smoker's cough to gross disability of breathlessness and pulmonary insufficiency. In other words, *bronchitis does not kill without the complication of emphysema.*

PULMONARY REACTIONS TO AIR POLLUTION DEMONSTRATED BY FUME FIXATION[15]

H. B. Boren, Eric G. Comstock, J. H. Gast, R. R. Rue, and B. J. Blumenthal

Conventional morphologic methods of examining the lung have several defects. Lung tissue is distorted both by deflation and sectioning. Small samples of lung are obtained with little chance of selecting regions representative of the lung as a whole. Findings associated with a localized abnormality are usually excluded by thin microscopic sections.

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[15] From the Veterans Administration Hospital, Houston, Texas.

Inflation and fixation of lung with formaldehyde fumes (1) results in a rigid specimen with minimal distortion. Whole lung sections may be cut and surveyed in their entirety. No one thickness has proved ideal for microscopic preparations. Normal lung was best demonstrated by sections 300 to 900 microns thick, whereas the most severe lesions of emphysema required sections up to 4,500 microns to prevent loss of tissue remnants attached outside the plane of sectioning. The use of a vacuum during the staining procedures gave uniform selective stains.

Using this method, the morphologic lesions of pulmonary emphysema in the human and the effects of inhaled carbon pigment and nitrite in mice were studied. Carbon was chosen because of its similarity to the pigment having high incidence in emphysema and because of its easy identification in tissue. It has also the ability to act as a carrier from other agents. Nitrite was chosen because of its role in producing damage to human lungs. Its concentration was determined colorimetrically (2).

To expose mice to NO_2 and carbon, two exposure systems were devised which operated at near atmospheric pressure.

From 10 to 30 mice at a time were exposed to carbon in air for six-hour periods. Concentrations of carbon of 16,000 per cc. were obtained, 38 per cent of the particles having a diameter of less than two microns. Lots of 5 to 20 mice were exposed to prepared concentrations of nitrite from 50 to 1,000 parts per million in air for thirty-minute periods.

Other methods used to study mouse lungs were viable bacterial counts of whole lung homogenates and biochemical determinations of total lung protein, total alpha amino nitrogen (3), and leucine amino acid peptidase (4).

In human necropsy material, morphologic evidence of emphysema was present in 41 of 44 male patients, although only 9 patients had clinical manifestations of emphysema. Pigment was usually present in areas demonstrating emphysema but absent in adjacent normal areas. The alveolar pore, defined as a circular or nearly elliptical opening of greatly varying dimension passing through an alveolar wall, was a constant finding in all stages of emphysema. Such pores were usually absent in adjacent areas of normal lung. The size and frequency of pores increased as the degree of emphysema increased but showed no relationship to the age of the patient. Adjacent pores tended to coalesce, and the boundary of all pores was reticulin. Within the edge of the pore, mucus, histiocytes, erythrocytes, and cellular debris were frequently found. Areas adjacent to pores showed increased numbers of histiocytes, round cells, and pigment, as well as localized areas showing abnormal staining reaction.

Morphologic examination of the lungs of 53 mice exposed to carbon showed the particles to be distributed throughout the entire lung. The amount of pigment increased as exposure time was lengthened. Within forty-five hours carbon was found in large clumps within the lung. Animals sacrificed at various periods after comparable exposures showed less carbon as the period after exposure increased. However, carbon was incompletely removed as long as ten months after exposure.

Quantitative viable bacterial counts of lung homogenates done on mice after carbon exposure showed a transient rise above a control level for twenty-four to forty-eight hours.

Except for occasional unexplained deaths in the exposure chamber, all animals appeared well, and no morphologic lesions were found.

A total of 190 mice were exposed to nitrite, the LD_{50} being in the range of 250 to 500 parts per million. More active animals succumbed to lower concentrations, and 100 per cent oxygen given after exposure decreased the mortality.

All mice manifested pulmonary distress during and following exposure, with death within twenty-four hours, or apparent recovery. There were no late manifestations during observation periods up to five months after exposure.

Comparisons of lung weight for a given mouse weight showed a large increase following nitrite exposure (320 per cent). Biochemical analyses showed a decrease of total protein, saline insoluble protein, free amino acids, and leucine amino acid peptidase with an increase of saline extractable protein. A slight transient increase of viable bacterial counts of whole lung was found. Morphologic examination demonstrated the gross findings of pulmonary edema and anoxia, which resolved in surviving animals within five to seven days. Evidence of bronchiolar inflammation was found, but there were no lesions of emphysema or alveolar pores.

Only characteristic gross alterations of lung tissue were accepted as morphologic evidence of emphysema. The changes seen are best described as a loss of alveolar tissue which results in *departitioning* of air space. This loss of tissue is associated with alveolar pore formation (5, 6). It is thought that the earliest change of emphysema is damage to some component of alveolar wall. With loss of this damaged tissue, alveolar pore formation results. As there is little associated fibrosis, a unique mechanism of removal of damaged tissue may occur. The form of the alveolar pore results in the interaction of tissue forces and surface forces. The net effect of surface forces, due to the

radii or curvature at the edge of the pore, causes most pores to continue to enlarge and to interfere with the capillaries bounding the pore.

Comparison of these observations of emphysema with those of carbon nitrite exposure shows large amounts of pigment associated with lesions in the human, but no lesions after exposure to carbon particles. Exposure to nitrite is an excellent method of producing pulmonary edema but the effects are widespread throughout the lung. Exposure to either carbon or nitrite may be associated with transient interference with bacterial clearing mechanisms, but this factor in itself had no appreciable effect. However, exposure of animals to carbon upon which an agent such as nitrite is absorbed may provide information as to the mechanism by which local damaging effects of an air pollutant are produced.

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RAPID FREEZING OF THE LUNG IN LIVING ANIMALS[16-17a]

Norman C. Staub and Winifred F. Storey

To achieve a real understanding of the function of the lung it is essential that we be able to relate moment-to-moment physiologic events in the lung to structural changes. We believe we have a powerful new tool for attacking this problem.

We open the thorax of an anesthetized animal under positive pressure breathing and, while continuously recording physiologic data either from the ventilatory or vascular phases of the lung, we freeze the lung very rapidly by pouring up to 3 liters of liquid propane (precooled in liquid nitrogen at −196°C.) over a selected portion of it. This procedure freezes the lung surface instantly, 0.1 cm. deep in 500 msec. and 0.2 cm. in 2,000 msec. A frozen portion is rapidly excised and transferred to absolute alcohol and corrosive mercuric chloride ($HgCl_2$) at −60°C. in a deep freeze for dehydration and fixation by freeze-substitution. When completely fixed, the specimen can be warmed to room temperature, imbedded in nitrocellulose, sectioned, and stained.

The specimens studied thus far show excellent gross and histologic preservation. They stain very well. Ice crystal size is submicronic because we achieve such rapid freezing. At present we confine our studies to the outer 0.2 cm. Although that is not the entire lung, it is a great deal more than can be obtained by any other method.

In our initial study we have been able to determine the relative ventilation of alveolar ducts and alveoli, respectively, in the cat lung during ventilation at 8 cycles per minute.

The importance of our method lies in the possibility of obtaining quantitative morphologic data and relating them directly to observed physiologic events. It seems to us that this offers many new approaches to the experimental study of lung physiopathology.

[16] From the Cardiovascular Research Institute, University of California Medical Center, San Francisco 22, California.

[17] Supported in part by U. S. Public Health Service Grant H-4029.

[17a] Presented at the Conference as an exhibit.

CORRELATION OF POSTMORTEM FUNCTION AND STRUCTURE IN NORMAL AND EMPHYSEMATOUS LUNGS[18, 19]

Philip C. Pratt

The demonstration was in two parts: first, the methods by which the compliance, residual volume, total volume, and spontaneous expiratory flow rate could be measured in excised lungs; and, second, a comparison of the functional and structural findings in normal and emphysematous lungs.

The methods used for post-mortem functional studies will be published elsewhere shortly and cannot be described here. Suffice it to say that the residual volume as determined in this investigation was the volume of air remaining in a lung

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[19] Presented at the Conference as an exhibit.

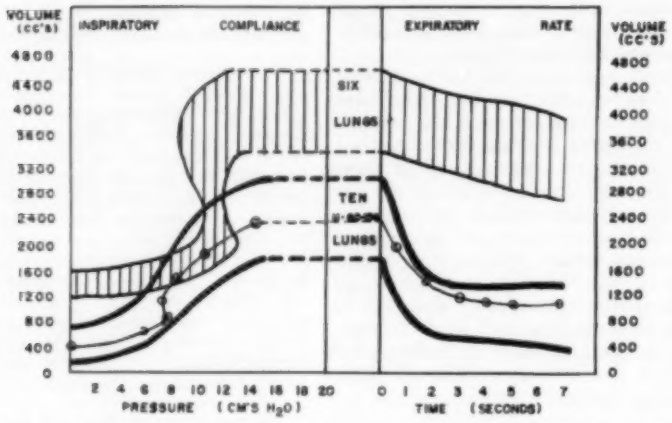

FIG. 1. Composite chart showing residual volumes (points of origin at left), inspiratory compliance curves, total lung volumes (highest point), and expiratory flow rates (right) for the largest and smallest of the 21 normal and 7 emphysematous lungs studied to date. (For interpretations, *see* text.) The curve for the individual lung (U 60-156) reveals a detail too involved for description here.

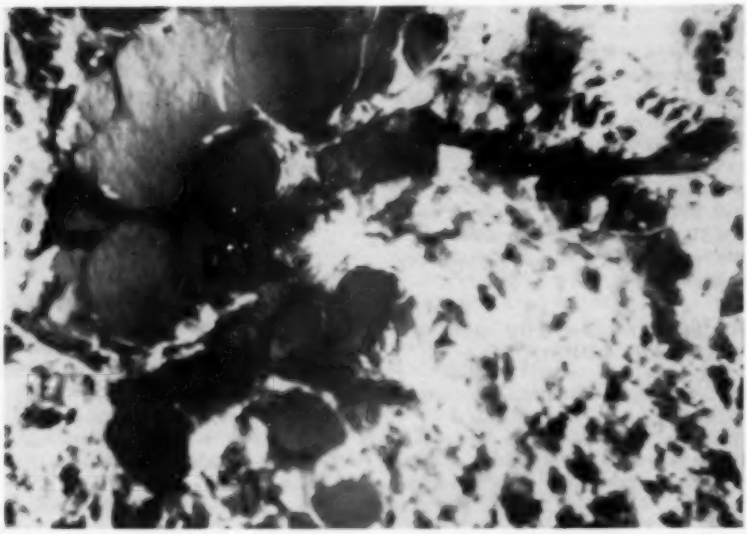

FIG. 2. An emphysematous area in a severely diseased lung. A bronchiole enters the field at the upper right and extends toward the left. It opens into a wide alveolar duct which clearly communicates with the emphysematous lesion. The bronchus has been traced proximally to the hilum of the lung and is patent throughout. (20×)

after ten minutes of free collapse from the fully inflated state. The total volume was the sum of the residual volume plus the total amount of air which the lung could be made to aspirate when exposed in a respirator box to negative pressure of a degree determined to be proper for each individual lung, usually between 10 and 15 cm. of water. The inspiratory compliance curves were obtained by recording separately the changes in pressure within the respirator box and the changing volume of the lung during a dynamic inflation of the lung. The pressure was then plotted against the volume. The expiratory flow rate curve was simply the spirometer tracing of the deflation of the lung from full inflation following sudden release of the "negative pressure."

The accompanying graph (figure 1) shows a comparison of all findings as to functional activity obtained to date. For clarity, only the largest and smallest of the 10 normal and 6 emphysematous lungs have been plotted. Each plot begins at the left at the level of the residual volume of the individual lung. Each curve extending from this point represents the dynamic inspiratory compliance of the lung. The reverse or S-shaped bend in the curves for emphysematous lungs was consistently present. Note that the emphysematous lungs reached their maximal volumes at lower pressure levels than those required for normal lungs. The highest point plotted for each lung represents its total volume, and this is consistently higher for emphysematous lungs than for normal lungs. Expiratory flow rates are plotted at the right. Note the difference in contour of the curves obtained with emphysematous as compared with normal lungs.

Following the functional tests the lungs were fixed with formalin vapor, dried in inflation, serially sectioned, and examined with a dissecting microscope. Tissue slices were available for examination with a microscope at the Conference. In essence, the significant point demonstrated was that, in cases of clinically recognizable emphysema with functional abnormalities established (including obstruction to airflow during expiration), it was possible to show anatomically patent bronchioles and alveolar ducts communicating with emphysematous areas (figure 2). The observations were interpreted as indicating that the primary abnormality in obstructive emphysema occurs in the alveolar walls rather than in the bronchioles. Thus, emphysema is considered to be a disease of alveolar septal tissue rather than the result of mechanical trauma caused by bronchiolar obliteration and air trapping, as currently believed by many observers.

ALVEOLAR DUCTS IN EARLY EMPHYSEMA: THEIR VESSELS, ALVEOLI, AND SOOT DEPOSITS[20,21]

CHARLES ODERR

I. *Observations bearing on the theory of alveolar duct functions and their inhibition by "soot" deposits.* The bulk of the lung is made up of respiratory units composed of alveolar ducts and their associated alveoli. Alveolar ducts are helix-like structures and the alveoli are radially arranged alcoves which "open" as the helix expands and "close" as the helix tends to collapse.

The number of the radially arranged alveoli appears to be determined partly by the species of animal and partly by the diameter of the ductus to which they belong. The most frequent numbers per 360° for man appear to be seven to nine.

One of the points of soot deposit is on the walls of the alveoli. Since these "soot stained" walls are frequently found in various stages of degeneration and destruction, it is a reasonable hypothesis that such loss of wall surface is one method by which "soot" reduces optimal lung function. Destruction in those walls shared by alveoli belonging to different alveolar ducts has still another deleterious effect of quite a different nature.

In order to explain this effect, it is necessary to consider the action of the alveolar duct respiratory unit during the course of breathing. If the boxlike shape of the alveoli during elongation and shortening of the ductus is considered, it will be seen that the volume of air contained in an alveolus could vary from near zero upward, as a parabolic function. The summit of the parabolic curve is reached when the "height," h, is equal to twice the "depth," d. The first part of the inflation curve is therefore very steep, but the volumes of air contained within the alveoli are relatively small. The last part of the inflation curve is very shallow so that, although the volumes of contained air are large, the *change* in volume (necessary for efficient exchange of gases) is small per unit of elongation or collapse of the ductus. The natural optimum is the middle part of the curve between these two extremes. Destruction of walls shared by alveoli belonging to different ducts creates the opportunity for gases to be shuttled back and forth between these peripheral units rather than to move efficiently in out-and-in directions. The ductus is thereby forced to operate in an excessively expanded (flattened) part of its parabolic curve.

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[21] Presented at the Conference as an exhibit.

A further effect of these "fenestrating wounds" might well be to accentuate the tendency of more proximal airways to collapse (Bernoulli effect?). Accentuation of this effect is also favored by "relaxation" of the helix-like musculature (*see* geodesic concept below). Such "relaxation" resulting in an increase in the diameter of the helix is an almost constant accompaniment of soot deposit. Increasing the diameter of the helix (at the expense of the depth of the alveoli) has several important results other than accentuating the Bernoulli effect, as follows:

The *volumes* of the ducts (representing conductance) and the alveoli collectively (representing blood-encompassed gas) may be considered to be proportional to the squares of their diameters. When the volume of the inner cylinder (ductus) enlarges at the expense of the outer cylinder (alveoli collectively), the ratio between the two volumes (normally believed to be about 1:2) changes drastically with very small increase in the diameter of the ductus.

The anatomic dead space represented by the alveolar ducts (inner cylinders) is not only very large but is nonstatic; it also changes with the segment of the parabolic-ventilation curve being utilized, and it increases drastically with small relaxations of the helix-like musculature.

These volumetric phenomena give rise to excessive dilution of the oxygen in the incoming air so that the partial pressure of oxygen presented to the absorbing surface of the alveoli is lower than normal.

Finally, the relaxation of the helix-like musculature from whatever cause would have the effect of destroying *geodesic* tension in the lung structure. The lost tension will be transferred to all surrounding structures, including the outer surfaces of the terminal respiratory bronchioles.

In this way a further accentuation of the Bernoulli effect could be brought about at this level of the respiratory tree during cough and other forceful expirations. Repeated Bernoulli action sets in motion the next cycle of peripheral (alveolar) change which eventually ends in bulla formations.

II. *Macroscopic observations bearing on the question of soot as a causative factor in emphysema.* 1. Whole lung sections of our material generally show only a small percentage of the total lung volume to be soot-stained. The manner of the gross parenchymal distribution varies, but in one pattern there are often hundreds of small areas of deposits a few millimeters in diameter. When these are examined closely in inflation-fixed lungs, it is seen that they involve a fractional part of a secondary lobule. The frequency with which early emphysema is found in this soot-stained part as compared with other parts of the same lobule shows a high correlation. There is a tendency for the diameter of the area of soot deposit to be larger than the macroscopically visible disease, but there is sometimes an interesting exception to this when the soot area is in the extreme periphery of the second lobule.

2. When the soot deposit is mild, a "preferred" trail from the center of the secondary lobule to an exit point at the periphery of the lobule is evident. The emphysema which develops along this path is normally inhibited at the periphery of the lobule, but it sometimes happens that a "kissing area" of disease develops in the adjoining lobule. This might suggest that noxious agents separated from the visible part of the soot may have affected the adjoining tissue.

3. It has been noted that many areas of soot-stained lung subjacent to intercostal spaces show localized emphysema. One explanation of this emphysema is that there is less chest wall support to combat the effects of cough. However, if it is assumed that emphysema developed independently of and previous to the soot, it is difficult to explain the correlation of soot and emphysema centrally when no localized unfavorable mechanical factor can be assumed. It seems logical to me to assume that mechanical factors are most effective when a chemical insult has preceded.

4. In a few rare instances we have run across apparently poorly vascularized secondary lobules, although they are fully aerated. It would seem, however, that they had been poorly *ventilated* over a long period of time because they contained much less soot than surrounding lobules, and likewise were free of the early emphysematous changes.

5. We think the absence of both soot and emphysema found in our dogs should be a clue for someone who is in a position to investigate various angles of the problem. It should be possible to determine whether their apparent immunity extends to higher concentration and to various kinds of "soot."

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INHALED ATMOSPHERIC POLLUTANTS IN THE GENESIS OF PULMONARY ABNORMALITIES[22]

ARTHUR J. VORWALD

All atmospheric pollutants in excessive quantities are obnoxious. In the light of present knowledge, however, only certain inhaled pollutants can be regarded as productive of significant toxic effects on the respiratory tract and lung. Consideration of those effects embodies a number of interrelated factors which usually exhibit a high degree of variability, but which, either alone or in combination, may operate to provoke functional and structural abnormalities.

From one point of view, the factors pertain to the pollutant.

1. It is obvious that the pollutant must be inhalable. Accordingly, the methodologies employed for collecting samples of the pollutant for study should assure that it is air borne at the breathing level of the exposed individual.

2. The atmospheric concentration of an inhalable pollutant partly determines the amount which gains access to the respiratory tract and lung. The importance of that concentration is obvious, but there is frequent failure to appreciate the significance of intermittently high or peak concentrations.

3. The particulate components of the pollutant must have a physical size sufficiently small to pass the filtering mechanisms of the upper respiratory passages, to enter the distal airways, and to become deposited in the alveoli. With respect to spheroid particles, primary attention is focused on those which are less than 5 μ in greatest diameter and especially on those having a size in the submicronic and ultramicroscopic ranges. Particles in the latter range are of special interest because of their possible biologic effect on the respiratory bronchioles, atria, and alveolar septa. In that regard, experimental studies have disclosed that rabbits, subjected to the prolonged inhalation of ultramicroscopic particles of free silicon dioxide, ultimately develop a diffuse chronic pneumonitis with attending emphysema and cor pulmonale.

4. The chemical nature of the pollutant has significance, not only with respect to its degree of purity, but also in regard to its quantitative and qualitative solubility in the cells and tissue fluids of the respiratory tract and lung. Furthermore, since the pollutant usually represents a combination of agents, it is important to know how one interacts with the other, possibly to form new agents or compounds or to induce synergistic, additive, antagonistic, neutralizing, or other modifying effects on the biologic system.

5. The primary pulmonary deposition, concentration, and retention of an inhaled pollutant, also its transport in and out of the lung, are determined in part by the pollutant's physical and chemical properties and by the nature of the biologic response which it provokes in different segments of the respiratory tract and lung.

6. The inherent biologic capacity of the inhaled pollutant to damage the pulmonary tissues is indeed a vital factor for consideration. Although other factors have been cited, they assume relatively less significance in comparison with the intensity and extent of the toxic action imposed by the pollutant in unit time.

From another viewpoint, there are equally important factors which pertain to the exposed individual. Those factors include the following:

The health status of the exposed individual—particularly as it relates to the respiratory tract and lung—either before, during, or after the exposure may influence the severity of the challenge imposed by the inhaled pollutant.

The individual's exposure should be scrutinized to determine whether it was intermittent or continuous, brief or prolonged, and whether it involved atmospheric concentrations of the pollutant, either in trace, low, high, or peak amounts.

The variable biologic response of different exposed persons requires that each be meticulously studied in the light of his past occupational and medical experience and of all pollutant factors which attended his exposure.

The clinical and post-mortem manifestations provoked by an inhaled pollutant depend not only on the anatomic but also on the physiologic and biochemical changes which it induces in different cells and tissue components of the respiratory tract, lung, and other organs of the body.

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THE REPARATIVE CAPACITY OF ANIMAL LUNGS AFTER EXPOSURE TO VARIOUS SINGLE AND MULTIPLE DOSES OF NITRITE[23]

JEROME KLEINERMAN AND GEORGE W. WRIGHT

Rats, rabbits, and guinea pigs were exposed to controlled concentrations of nitrogen dioxide for

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two-hour periods. In single-dose experiments, animals were subjected to either a high dose (rabbits 200 p.p.m.; rats 150 p.p.m.), a moderate dose (rabbits 100 p.p.m.; rats and guinea pigs 75 to 80 p.p.m.), or a low dose (rabbits 25 p.p.m.; rats and guinea pigs 15 to 20 p.p.m.), all for two-hour periods. Following this exposure, animals were sacrificed at one, two, four, seven, fourteen, and twenty-one days; the lungs were fixed in inflation, prepared, and studied histologically. In multiple-dose experiments animals were exposed on five successive days to low concentrations (15 to 25 p.p.m. for two-hour periods). Animals were sacrificed according to the above schedule. All rats and 33 per cent of the rabbits died with severe pulmonary edema within twenty-four hours of the single high dose. All animals survived the single moderate dose. At twenty-four hours in these groups a moderate degree of pulmonary edema was observed. Inflammatory exudate was most prominent in the region of the respiratory bronchioles, and epithelial degeneration and regeneration were noted. By the fourth day, edema and acute inflammatory changes were replaced by macrophage infiltration, and epithelial regeneration was intense, localized mainly in the region of the respiratory bronchiole. Repair was almost complete by two weeks; only traces of chronic inflammatory exudate remained and these were usually perivascular; some epithelization of alveolar spaces about respiratory bronchioles also persisted. This pattern of repair was seen also in the animals receiving lower doses of nitrite, but the edema, epithelial desquamation, inflammation, and epithelial repair were of lesser severity, being roughly proportional to the concentration of gas to which the animal was exposed.

In the multiple low-dose exposures, edema and inflammation were less severe in an early stage, but peribronchial and perivascular chronic inflammation were more pronounced in the rat and guinea pig. The number of respiratory units severely involved was less, and epithelial regeneration was not prominent. Repair appeared to be almost complete by seven days after exposure, but traces of inflammation persisted.

In summary, permanent alteration was not observed in the lungs of animals surviving exposure to nitrite in the doses mentioned. Pulmonary edema was observed early and inflammatory and epithelial regenerative changes were most severe in the region of the respiratory bronchiole. Healing appeared to be practically complete by two weeks after the insult.

AUTOCATALYSIS OF PULMONARY EDEMA[24]

PAUL GROSS, MARIAN L. WESTRICK, AND JAMES M. MCNERNEY

Irritating air pollutants tend to produce pulmonary edema. This may be focal and minimal or widespread and severe, depending upon the degree of irritation produced.

It is our concept that minimal pulmonary edema may trigger an autocatalytic mechanism in the presence of reduced pulmonary reserve, such as severe emphysema, or in the presence of pneumoconiosis which may terminate as a fatal, widespread, pulmonary edema.

In the case of reduced pulmonary reserve, the presence of focal edema is believed to cause focal anoxia which tends to produce more edema. In the case of pneumoconiosis, focal edema will mobilize and disperse stored dust in that region. This liberated dust, being an irritant, will produce more edema which, in turn, liberates more dust.

In order to test the applicability of this concept, the following experiments were devised.

The pulmonary reserve was reduced by 40 per cent in 90 rats by the production of oleothorax with 10 ml. of mineral oil.

Two other groups of 90 rats each were given multiple intratracheal injections of quartz dust and kaolin dust, respectively, over a period of six months for a total dosage of 150 mg.

A group of 30 normal rats were given 6.4 mg. α-naphthylthiourea (ANTU) which produced pulmonary edema, causing 27 per cent of the animals to die. The same dosage of ANTU administered to the oleothorax and pneumoconiotic rats caused mortalities of about 60 per cent in the different groups regardless of the nature of the handicap.

Microscopically there were significant changes in the structure of the dust foci. The normal clay dust deposit was sharply delimited as was its reticulin framework. The dust content was moderately heavy. The quartz dust deposit was similarly sharply outlined with a reticulin framework in which the fibers were of uniform thickness and staining character. The dust content was heavy and was sharply delimited by peripheral membranes.

In the presence of edema, there were significant structural changes in the dust foci. The outlines were hazy; the cells were widely separated. There was a predilection of edema fluid for alveoli surrounding dust deposits. The stromal framework of the foci showed irregular condensations and frag-

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mentations as well as enlarged spaces of various sizes. The dust content was greatly reduced and there was much dust widely distributed throughout much of the edema fluid.

Some of the dust foci in animals that had recovered from the ANTU injections were completely devoid of dust.

In summary, we have found that a given dose of ANTU which killed 27 per cent of normal rats due to pulmonary edema, will kill about 60 per cent of rats handicapped either by a reduced pulmonary reserve or by pneumoconiosis. It makes little difference whether the pneumoconiosis is caused by a fibrogenic dustlike quartz or a nonfibrogenic dust such as kaolin.

Microscopic study of the edematous pneumoconiotic lungs revealed significant changes in the structure of the dust foci. These changes were associated with a partial demineralization of the latter and a wide dispersion of mobilized dust particles throughout much of the intra-alveolar fluid.

These findings plus the observed predilection of intra-alveolar edema for the regions around dust deposits are interpreted to support our concept that, in the presence of pneumoconiosis, a relatively small amount of pulmonary edema may become autocatalytic and terminate fatally. A similar autocatalysis of pulmonary edema is believed to occur when the pulmonary reserve has suffered significant reduction.

PATHOLOGIC CHANGES IN THE ELASTIC TISSUE AND BRONCHI IN CHRONIC PULMONARY EMPHYSEMA[25]

ROBERT R. WRIGHT

Chronic obstructive emphysema has been shown to be associated with changes in the elastic behavior of the lung. This is generally reflected by: (*1*) a reduced intrapleural negative pressure which indicates a loss of elastic recoil (1); (*2*) a premature closure of the small airways during the expiration of air due to a reduction in the elastic radial traction on the walls of the small airways (2); and (*3*) changes in the patterns of the pressure-volume curve of the inflated lung (3).

The fine structural framework of the lung can be best appreciated when studied in its three-dimensional state. A new technique was developed for the tridimensional microscopic study of elastic tissue in the lung.

Fresh autopsy and surgical lung specimens were infused with Zenker's solution through the bronchi. After twelve hours of fixation, thin sections of lung tissue (200 μ to 300 μ) were cut, de-Zenkerized, stained with orcein elastic stain, mounted on microscopic slides, and studied with the conventional light microscope.

The elastic fibers of the normal lung form a complex interlacing framework throughout the lung and contribute a prominent structural component to the airways, alveoli, vessels, septae, and pleura. The bronchi possess a continuous layer of longitudinal and circular fibers, which develop into bundles at the level of the respiratory bronchiole and continue as prominent bundles in ringlike formations which comprise the alveolar ducts and the mouths of the alveoli. From these bundles fine strands extend onto the walls of the alveoli where they unite with other fibers arising from nearby similar elastic bundles. There is a continuous exchange of elastic fibers between the air passages, alveoli, vessels, and septae, thus composing a pulmonary "elastic continuum" (4).

There were striking derangements in the elastic fiber framework of chronic emphysematous lungs. The elastic bundles of the alveolar ducts and alveoli were fragmented, frayed, and in some areas completely absent. At the sites of interruption, the bundles occasionally revealed a coiling of the retracted ends of the individual fibers. The areas showing severe elastic tissue deterioration were associated with deformities of the airways and alveoli consisting of overdistended alveolar ducts, effacement of alveoli, and fenestration to complete loss of interalveolar septae, all of which produced the enlarged air spaces morphologically characteristic of chronic emphysema.

Centrilobular emphysema of a mild degree unassociated with any known pulmonary functional disturbances was a common finding in essentially normal lungs. This focal emphysematous degeneration confined in the mild forms of the disease to the central zone of the secondary lung lobules is nearly always associated with the focal deposition of air-borne black anthracotic pigment. At the sites of pigment deposition, the elastic fibers were observed to be in various stages of deterioration. Frequently elastic fibers were completely absent in those areas. Associated with these defective elastic fibers was an overdistention of the involved air passages. This pathologic change occurred most commonly at the junction of the respiratory bronchioles and the alveolar ducts, which corresponds to the centrilobular location. Frequently, there were mixtures of the centrilobular and the diffuse types of emphysema, and severe elastic fiber de-

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terioration was widespread. Usually, however, even in the most severely advanced stages of chronic emphysema, focal areas of intact lung with normal-appearing elastic fibers could be found.

A separate study of the bronchial trees of 20 normal and 20 severely emphysematous lungs of patients in comparable age groups was made for comparison (5). The lungs were infused with formalin solution, fixed for forty-eight to seventy-two hours, and then the bronchial trees were dissected free of surrounding tissue to within 3 to 4 cm. of the periphery of the lung. Obvious differences between the bronchi of the normal and the emphysematous lungs were observed. These consisted of a softening, thinning, and atrophying of the bronchial walls of the emphysematous lungs involving the medium-sized bronchi, occasionally the larger bronchi, and less often the major bronchi and trachea. These airways were more easily collapsed than the normal by manual pressure, and the medium-sized bronchi frequently appeared as flaccid ribbons rather than tubular bronchi. Microscopically, there was a loss of cartilage, smooth muscle, and connective tissue in the walls of these bronchi. Evidence of active inflammation was minimal other than the occasional acute terminal suppurative bronchitis, which was considered to be a terminal complicating event and not of pathogenic significance.

These pathologic changes in the elastic tissues and bronchi may explain some of the functional impairments in ventilation that occur in chronic obstructive pulmonary emphysema. The normal lung is in a continuous, although altering state of inflation throughout life, due to the fact that the semirigid chest wall and the airless intrapleural space prevent the elastic forces of the lung from producing a collapse of the organ. Thus, the lung is maintained in a state of stretch, and the lung tension, due to the elastic properties of the lung, is maintained within certain limits despite the changing volume which occurs with ventilation. This elastic tension provides adequate radial traction on the small airways and alveoli for the prevention of collapse. The destructive changes observed in the elastic tissue in chronic obstructive emphysema alter the elastic properties of the lung. There would reasonably be a lessened radial traction on the small airways when the lung was less than fully inflated, thus allowing the air pressure gradient which exists between the outside and the inside of the intrapulmonary bronchi to collapse the small airways during exhalation of air, especially during a forced expiration. This premature closure of the airways would produce an expiratory airway obstruction and an increased residual volume within the lung, which is characteristic of chronic obstructive emphysema. The atrophy and increased ease of collapse of the bronchi in the severely emphysematous lung would appear to enhance the obstructive phenomenon in that the medium and large bronchi would collapse, in addition to the small airways, during exhalation.

The cause of the breakdown in elastic fibers and the atrophy of the bronchial tree in chronic obstructive pulmonary emphysema could not be determined from these studies. The relationship between the two observations as far as etiology and pathogenesis is concerned also remains to be explained. The interesting association between the centrilobular form of emphysema and air-borne particles may indicate a direct form of lung damage due to air pollution.

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THE MORPHOGENESIS OF PANLOBULAR EMPHYSEMA[20]

John P. Wyatt and Herbert Sweet
(With the technical assistance of
Vernon W. Fischer)

Contemporary interest in emphysema has been largely concerned with sophisticated pulmonary function tests which measure ventilatory capacity, gas distribution and diffusion, and intrapulmonary blood flow. Although from a morphologic viewpoint numerous analytic methods for the evaluation of structural changes, such as Gough paper sections, corrosion casts, and stereoscopic microscopy, are available, the pathologic study of pulmonary hyperinflation has lagged far behind clinical investigation.

One characteristic pattern of generalized emphysema, recognized earlier on morbid anatomic evi-

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dence, involved the central portions of Miller's secondary lobule with the lesion of primacy being at the level of the respiratory bronchioles (centrilobular emphysema). A second characteristic form of emphysema, equally as common as the centrilobular form but with the emphysematous lesions being diffuse throughout the secondary lobule due to overdistention and a concurrent dissolution of the reticular tissue of the respiratory lobular tissue, is now identified and analyzed in detail in this essay.

A study of the early and progressive structural changes which are responsible for this distinctive form of generalized emphysema, as well as the important changes which occur at the arteriolar-capillary level, greatly contribute to our knowledge concerning the initiation, evolution, and clinical findings of this distinctive pulmonary disability.

Material and methods: Ninety-eight cases of panlobular emphysema were analyzed. A battery of techniques were applied to these cases, many of which had had numerous antemortem function studies. After removal of the heart-lungs as a functional bloc, postmortem bronchoangiographic and spirometric studies were first carried out. The ventricular muscles and septa of the hearts were separated, defatted, and weighed. These weights are correlated with the extent of emphysema. After controlled inflation of the lung with formalin, multiple 1-inch whole lung gelatin-infiltrated frozen blocks were used for orientation purposes. Gough whole lung paper section, 125 micron frozen sections for capillary, elastic, and reticular studies, stereoscopic observations, histologic serialization of the secondary lobular lesion, and corrosion casts were complemental techniques utilized.

Macroanatomy of panlobular emphysema: Panlobular emphysema is a common and distinctive form of pathologic distention and dissolution of the terminal air ducts and sacs. This change is uniform and diffuse throughout the secondary lobule. Depending upon its anatomic distribution in the lung field, this genre of emphysema may be confined either to a segment or lobe of a lung or is widespread throughout the whole lung field. As these localized patterns are congeners of the generalized (widespread) type, both macroscopic and microscopic lesions are included under a composite description.

Scanning the whole lung, paper parchment revealed, as the most prominent aspect, an even, diffuse overdistention and rupture of the terminal respiratory tissue (figure 3). Under the stereoscope, most "alveolar fields" measured between 180 and 240 μ, and the respiratory bronchioles in the central portions of the lung showed a limited increase in caliber. With rupture of air sac walls, uniform microbullous spaces with dimensions of 500 μ or greater were formed (figures 4 and 5).

A second prominent marking in all generalized forms of panlobular emphysema was that the emphysematous state was much more advanced in the lower lobes of the lungs. This is in contrast to generalized centrilobular disease in which the major concentration of disease is usually in the upper half of the lung.

A third distinctive feature which separated panlobular emphysema from the centrilobular form was the limited degree of carbon pigmentation and its diffuse distribution throughout the affected lung (figure 4), in contrast to pigment agminations around the damaged respiratory bronchioles of centrilobular emphysema. In the absence of pigment, the lung had a blanched or leeched appearance on the paper parchment, a natural color often observed in the lungs of the aged, although the majority of patients with panlobular emphysema were less than sixty-eight years old.

A fourth characteristic aspect, most prominent in the moderate and advanced stages of panlobular emphysema, was the numerous round, oval, or elliptical lacunae in the abnormally stretched air sac walls. These alveolar wall fenestrations, well described by Loeschke, have sharply delimited borders, often associated with black pigment spread over the surface of the alveoli.

With the whole lung sections used as a screening procedure, the constancy of panlobular emphysema, confirmed by stereoscopy, was associated in 60 per cent of the cases with major lung or thorax cage disease.

In 6 cases the patients had marked primary kyphoscoliosis with boxlike chests. In 3 cases of "congenital" lobar emphysema in children, aged one year, five, and eleven years, the constant finding was the presence of uniform lobular hyperinflation, with bullous fields in the lungs of the five- and eleven-year-old children. A third clinical group, which showed a moderate degree of panlobular emphysema, was composed of three males, aged fifty to fifty-two, in whom an occlusive type of laryngeal cancer had been clinically present from twelve to fifteen months. A fourth group was composed of heterogenous pathologic conditions producing hilar bronchial root compression. There were 25 examples of localized large bronchial disease, due either to the presence of endobronchial and peribronchial silicotic scar tissue at the lung hilum, a similar process attributable to tuberculosis, or to combinations of both of these pathologic conditions. There were 9 examples of bronchial root carcinoma with regional lymph node metastases. A feature of most of these neoplastic examples was bronchial wall impingement by greatly enlarged hilar lymph nodes with com-

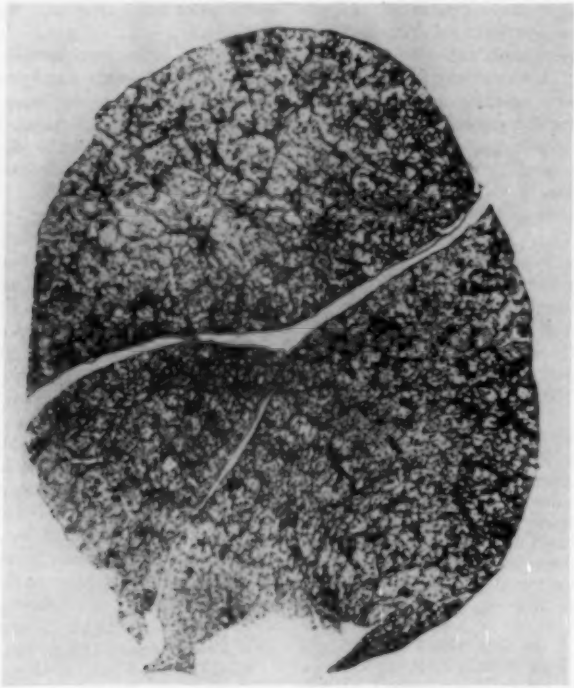

FIG. 3. Whole lung section mounted on paper parchment reveals an even diffuse overdistention and rupturing of the terminal respiratory tissue: panlobular emphysema.

pression of the larger bronchi producing indentations and geniculations. In 2 cases of interstitial lipoid pneumonia, in which a large degree of scarring was associated with the peribronchial tissue, segmental patterns of panlobular emphysema were observed. With tridimensional studies the pattern of emphysema showing air sac distention with dissolution was found to be similar in all types of localized bronchial narrowing.

In a number of cases of extensive emphysema in which there was no evidence of sclerotic bronchial disease or alterations of the thoracic skeleton, there was a diffuse extensive bronchiectasis demonstrated by postmortem bronchograms.

The contrast between these markedly ectatic large bronchi and those observed in the control material was striking. Although extensive bronchographic abnormalities of a diffuse nature were encountered in a substantial number of examples of generalized panlobular emphysema, the bronchial wall deformity was best demonstrated in the advanced cases.

A greater number of cases were shown to have normal postmortem bronchograms. The degree of emphysema associated with this larger group was not as pronounced as that seen in those with abnormal bronchograms.

Micro-anatomy: In the earliest cases, analysis of the serial sections revealed a uniform dilatation from primary lobule to primary lobule, including air ducts and their air sac derivatives. A common appearance was the ironing out of the air sacs, creating a shallow basin for each alveolus. The respiratory bronchioles of the second and third order shared in this overexpansion. The elastic tissue appeared to be thickened nearest the ductal mouth with disappearance of the elastica and the reticulum from the bases of the air sacs. This latter finding was particularly noticeable in the early to moderate forms of panlobular emphysema. The alveolar basins appeared to be the commonest site for sac rupture, destruction, and the appearance of alveolar fenestrations. If black pigmentation was noted in panlobular emphysema, it was present in its greatest concentrations over the air sac walls.

In those specimens in which the bronchogram and anatomic dissection revealed a diffuse ectasia and multiple sacculations of the large bronchi, the

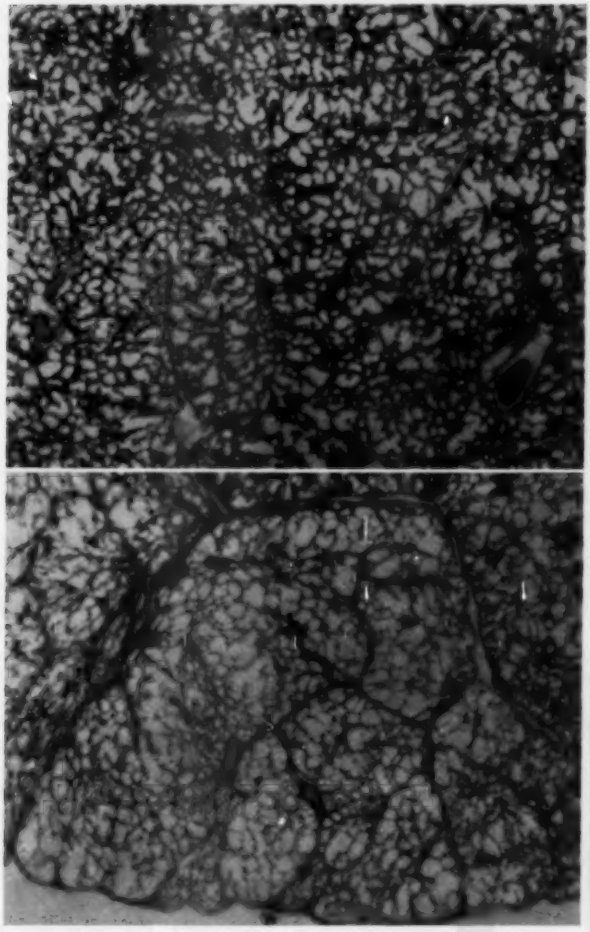

FIG. 4 (Upper). The same as figure 3 under higher magnification.
FIG. 5 (Lower). The same as figure 4 showing microbullous spaces measuring 500μ or more in diameter.

outstanding change was the presence of mucous gland hyperplasia, almost adenomatoid in degree at these sites. The mononuclear cell response was spread between muscle components and elastic tissue and around glands. In between the cartilage and around the gland-bearing regions there was atrophy of the associated musculature.

Pulmonary vasculature lesions: Total density of these vascular casts in generalized panlobular emphysema indicated a generalized denudation of the arterial and arteriolar channels throughout the lung field. In the most advanced cases of emphysema, usually 20 to 30 per cent of the lung cast appeared to have the normal architecture and vascular preservation, usually in certain anatomic regions such as paravertebral or posterior apex (figure 6).

A second feature recorded was a local redundancy of the pulmonary venous bed in a few of the advanced examples of emphysema. Multiple focal spider-like congeries were found throughout the casts and were traceable back into main pulmonary venous channels.

Correlation of right heart weights with the anatomic extent of emphysema revealed in those with advanced degrees of emphysema a significant

FIG. 6. Pulmonary vascular casts from diffuse panlobular emphysema (left) and normal (right) lungs.

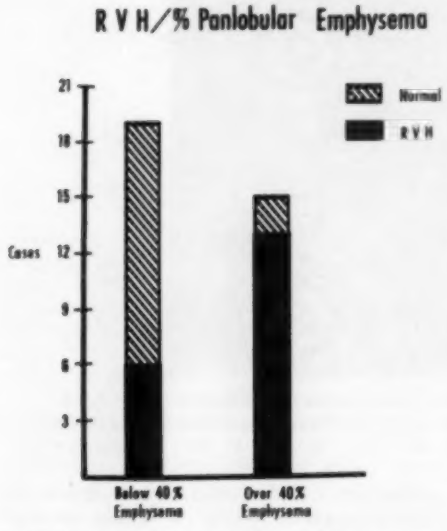

FIG. 7. Correlation of weight of right hearts with degree of panlobular emphysema.

increase in the number of cases with right ventricular hypertrophy (figure 7). This finding suggests that the development of right ventricular hypertrophy is a relatively early phenomenon, with a progressive thickening of the right ventricle closely associated with the gradual increment in "pathologic dead space." It would appear that the compromisation of the capillary bed throughout the secondary lobule is much more readily accomplished and observed earlier in panlobular emphysema than in the centrilobular type.

Spirometric analysis: Postmortem inflationary and deflationary studies revealed in generalized panlobular emphysema a lag in both inspiratory and expiratory phases. These two features can be observed on a sample tracing, compared with controls from the same age group. Flow rates in panlobular emphysematous lungs averaged about half the flow rate shown by normal lungs inflated to the same volume. The pressure of the diseased lungs at maximal inflation was the same as normal, but resistance to flow was three to ten times that recorded in normal lungs.

Discussion: Panlobular emphysema is not a new disease entity. This distensive-dissolutive form of emphysema represents that variant of pulmonary hyperinflation referred to in earlier clinical literature as chronic vesicular emphysema or the soufflé lung. But its recognition and progressive functional derangements are more clearly understood if the structural concept of the secondary lobule of Miller is followed, not only in terms of respiring lung tissue, but also the intimate capillary pattern associated with the air ducts and air sacs.

Pathologic conditions associated with panlobular emphysema: In 6 cases of idiopathic kyphoscoliotic deformity in adults, including 4 females and 2 males, their respective ages being thirty-eight, sixty-two, sixty-seven, seventy-two, and forty-one and fifty-seven, grid analysis revealed that the "pathologic dead space" estimation was not above

grade II (up to 40 per cent). The limited degree of pulmonary hyperinflation indicated that the right ventricular hypertrophy found in the hearts of these subjects was probably due to vascular root distortion produced by the gibbous thorax, rather than any capillary occlusion attributable to the limited air sac distention and rupture. The explanation for this moderate type of panlobular emphysema probably may be due to the thorax being fixed in the midinspiratory position. This thoracic cage distortion contributes to impairment in pulmonary ventilation or lung toilette followed by a limited increment in residual volume and secondary loss of reticular tissue resulting in permanent air sac distention and rupture.

A moderate degree of generalized emphysema, involving all five lobes of the lungs equally, was noted in 3 cases of cancer of the larynx. Although a cause-and-effect relationship cannot be unequivocally established in these 3 cases, the association between laryngeal narrowing and this form of pulmonary overdistention appears significant.

Permanent air sac distention is a unique phenomenon in the early years of life, but it is found and may be associated with bullous areas in a rare clinicopathologic state called congenital lobar emphysema. In our 3 cases, the anatomic type of emphysema associated with proximal bronchial cartilagenous derangements was that of the panlobular type.

From scrutiny of the literature, the constant type of emphysema observed in these affected lobes or lungs is that of overdistention-dissolution of air ducts and sacs. In the late stages of the process there has been structural damage of lung acini, progressing to bullous field formations.

The constancy of the finding of panlobular emphysema with pathologic processes such as localized silicosis and tuberculosis, primary bronchial cancer, or fibrosing lipid pneumonia, each process revealing bronchial narrowing, has focused attention upon the importance of extrinsic and intrinsic disease of the large bronchi in the production of air-flow disturbance. It has been suggested that the deforming hilar sclerosis produces local nerve demyelination and thus interferes with bronchial wall dynamics.

The development of widespread lobular emphysema without associated pathologic conditions as enumerated above and without a significant degree of bronchiolar narrowing or obliteration suggests that the lobular distention and dissolution may also occur as a primary disturbance, without mediating influences such as bronchial airway clearance. The uniform yielding of primary lobules may be a basic reaction of injury at the air-membrane level. That inhaled material can induce generalized focal emphysema is well known, coal being the agent responsible for the progressive emphysematous syndrome as observed in coal workers in Cardiff and southern Illinois. In nonindustrial centrilobular emphysema, the location and concentration of carbonaceous material at the respiratory bronchioles may be directly or indirectly responsible for the type and extent of that form of emphysema.

In panlobular emphysema, the deep penetration of dust particles into the ultimate reaches of the alveolar ducts and alcoves may be one factor responsible for air sac dissolution interfering with the intrapulmonary dynamics recognized by the clinical manifestations of air trapping and perfusion difficulties.

(*To be concluded in the April issue of the* REVIEW)

Notes

RISK OF TUBERCULOUS INFECTION AND OF RADIATION AMONG UNIVERSITY STUDENTS[1]

A world-wide concern about tuberculosis has existed for centuries. Many decades have elapsed since discovery of the tubercle bacillus and for several decades medical workers have made great efforts to reduce the hazard of tuberculous infection by prevention and early detection. The recognized modern and economical way to eliminate the risk of tuberculous infection and facilitate early detection is by skin testing and roentgenographic examination. These public health methods lead to the other world-wide concern, which has been alarming mankind increasingly for the last decade, namely, the hazard of radiation.

It is our duty and task to find a solution for these conflicting risks, weighing them according to their importance on each occasion. It is necessary to find the most effective, economical, and innocuous way to protect the health of the university students, the future leaders of the nations, against both hazards in an age group which is most sensitive to these dangers.

It is impossible not to accept Wilson's[2] dictum that "We are going to have tuberculosis with us for another two generations." To paraphrase his statement, a 20-year-old student whose skin test converts to positive now may develop active tuberculosis and may infect his grandchildren when he is seventy.

While it is true that a complete *individual* examination should include a chest roentgenogram and a tuberculin skin test in private practice and in the hospital,[3] it is equally true that in a tuberculosis screening program the positive yield of microfilming is low in a population with a low infection rate.[4]

There is no doubt that the tuberculin test is by far the most important epidemiologic tool in early detection of infection.[5, 6]

Since we are dealing with a group which is at the beginning of its procreative years, it is exceedingly important to reduce the ionizing radiation effect (particularly that of gonad doses) to a minimum.[7] In this respect it is very reassuring that the usual dosage of radiation to the gonads is very low when using the new reflector lens optic system, the photofluorograph.[7, 8] According to Hodges[7]: "...with the possible exception ...in which the ovary or testis ...lies in the direct beam, diagnostic radiology, ...is not contributing 30-year gonadal doses that are significant relative to background" (radiation).

In a previous study,[9] the present writer found that the tuberculin skin test conversion rate of foreign-born students is statistically significantly higher than that of North American-born students. Further important findings were: the significantly lower conversion rate among the American (U.S.- and Canadian-born) white males than among the Negro male students, and the higher percentage of the conversion rate of medical students than that of the nonmedical student population. The study revealed that the tuberculin positivity increased among the foreign-born and decreased among the North American students during a seven-year (1948–1955) period. As a consequence of this information, the tuberculosis control program at the University of Chicago was radically changed in 1958.

For this purpose, the student population was divided into two groups: the first ("general risk") group consisted of the native white students, including the first- and second-year medical students. The nonwhite and foreign-born students, the workers in the field of tuberculosis, and the third- and fourth-year medical students belonged to the second ("special risk") group. At the entrance examination every student had been given a chest microfilm and a tuberculin (PPD, intermediate-strength) skin test, with the exception of previously positive reactors.

The skin test was performed with 0.1 ml. of tuberculin PPD intracutaneously on the flexor surface of the forearm and was read after forty-eight hours. It was considered positive if the induration was at least 5 mm. and the erythema at least 10

[1] This study was supported in part by the A. B. Kuppenheimer Fund.

[2] WILSON, J. L.: Returning tuberculosis to the family physician, Clinical Conference of the Chicago Medical Society, March 2-5, 1959.

[3] CUSTER, E. W.: Amer Rev Tuberc, 1959, *79*, 378.

[4] WOOD, L. E., *et al.*: Amer Rev Tuberc, 1958, *78*, 667.

[5] STEIN, S. C., AND HETHERINGTON, H. W.: JAMA, 1960, *173*, 129.

[6] MYERS, J. A.: Med Forum Modern Med, 1958, *26*, 32.

[7] HODGES, P. C.: JAMA, 1958, *166*, 577.

[8] PERKINS, J. E.: Tuberc Abstracts, 1957, *30*, 1.

[9] SZENT-GYÖRGYI, N.: Amer Rev Tuberc, 1957, *76*, 308.

TABLE 1

TUBERCULIN REACTIONS ACCORDING TO THE RISK GROUPS DURING TWO ACADEMIC YEARS (1958–1960)

Group of Students	Total		Negative		Converter		Positive	
	Number	Per Cent	Number	Per Cent	Number	Per Cent*	Number†	Per Cent
Total	4,192	—	3,729	88.9	68	1.7	463	11.0
General risk	3,393	80.9	3,190	94.0	31‡	0.9	203§	5.9
Special risk with medical students	799	19.0	539	67.4	37‡	6.4	260§	32.5
Medical students	83	1.9	71	85.5	4	5.3	12	14.4
Special risk without medical students	716	17.0	468	65.3	33	6.5	248	34.6

* Computed from the sum of negatives and converters.
† Converters included.
‡ $\chi^2 = 21.26$ $P < 0.01$.
§ $\chi^2 = 464$ $P < 0.01$.
Differences are statistically significant.

mm. in diameter. The skin-test solution was prepared in such a way that 0.1 ml. contained 0.0001 mg. of tuberculin PPD.

Microfilms only were obtained on the previously tuberculin positive students. Since it was learned from the writer's previous study[8] that the risk of infection is definitely and significantly lower in the American white student population than in the other student groups, it seemed reasonable to reduce the hazard of ionizing radiation on the native white group without increasing the hazard of tuberculous infection and without endangering the advantage of early detection. It was decided that in the follow-up examinations the "general risk" group should have skin tests and microfilms every three years only and the "special risk" group should have the same tests annually.

The results of the modified tuberculosis control program are encouraging enough to give assurance that the direction followed is correct.

The modified program was introduced in the 1958–1959 academic year when 1,708 University of Chicago students were skin tested in the way above outlined. In the year 1959–1960, 2,484 students were skin tested and the results are now available of 4,192 tests for the last two academic years.

All newly entering students were skin tested, excluding those with known tuberculosis, students with previous histories of tuberculosis, previously positive skin tests, and BCG immunization. In addition, the foreign-born, the nonwhite, and the third- and fourth-year medical students who had negative skin tests during the previous academic year were skin tested. Furthermore, the American white students who had not had skin tests within three years were included. (American-born whites constitute about 83 per cent of the student body at the University of Chicago.) Very many of the American white students had skin tests during the previous year, since the modified program was originated only in the fall quarter of 1958.

It was found (table 1) that 11.0 per cent of the total population were positive skin-test reactors. The native American white population ("general risk") of the students was represented by 5.9 per cent positive reactions in contrast to the 32.5 per cent positivity of the "special risk" group, which consisted of the foreign-born, the nonwhites, junior and senior medical students, and workers in the field of tuberculosis. The difference between the two groups is, statistically, highly significant.

If this finding is compared with the result[8] at the same University in 1955, the improvement is quite obvious. In the previous study, 17.5 per cent of the American whites, 43.5 per cent of the American Negroes, and 61.2 per cent of the foreign-born students were positive skin-test reactors. The percentual drop of the positive reactions between 1955 and 1960 (the present study) is very significant in each group.

Noteworthy is the percentage change in the relation of positivity of the American white and the foreign-born group. The improvement in the American group is more rapid than that in the foreign-born group. At the same university, Loosli and co-workers[10] found that the prevalence of positive reactions of the Americans was less than one-half that of the foreign-born in 1948 (24.8 per cent to 54.6 per cent, respectively). Seven years later the present writer[8] found that this rate was less than one-third (19.0 per cent to 61.2 per cent). In 1960 that rate is still changing in favor of the American-born white students, the cutaneous sensitivity to tuberculosis for the native whites be-

[10] LOOSLI, C. G., *et al.*: Amer J Hyg, 1951, *53*, 33.

TABLE 2

COMPARISON OF THE TUBERCULIN SKIN TESTS IN 1958–1959 AND 1959–1960 ACADEMIC YEARS

Group of Students	Total				Negative				Converter				Positive			
	1958–1959		1959–1960		1958–1959		1959–1960		1958–1959		1959–1960		1958–1959		1959–1960	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	1,708	—	2,484	—	1,541	90.2	2,188	88	25	1.6	43	1.9	167	9.7	296	11.9
General risk	1,358	79.5	2,035	81.9	1,289	94.9	1,901	93.4	9	0.6	22	1.1	69	5.0	134	6.5
Special risk with medical students	350	20.5	449	18.1	252	72.0	287	63.9	16	5.9	21	6.8	98	28.0	162	36.0
Medical students	66	3.8	17	0.7	56	84.8	15	88.2	4	6.6	0	0	10	15.1	2	11.7
Special risk without medical students	284	16.7	432	17.4	196	69.0	272	62.9	12	5.7	21	7.1	88	30.9	160	37.0

ing less than one-fifth that of foreign-born and of nonwhites (5.9 per cent and 32.5 per cent, respectively).

The percentage of positivity has decreased significantly from 17.5 to 5.9 per cent for the American whites since 1955.

The conversion from negative to positive skin tests for the two academic years (1958–1959 and 1959–1960) was 0.9 per cent for the "general risk" group and more than seven times higher (6.4 per cent) for the "special risk" group. The conversion rate was 5.3 per cent for the clinical (junior and senior) medical students, which is quite close to the figures for the rest of the "special risk" group (6.5 per cent) (*see* table 1). There was speculation concerning the reason for this great discrepancy of the conversion rate between the two groups, but no satisfactory explanation was found. Nevertheless, it is very reassuring that the number and the percentage of *converters* steadily and significantly decreased at the University of Chicago between 1955 and 1960. It proves that a consistently followed long-range tuberculosis control program, with the necessary and possible flexibility, is rewarding and worth while.

The higher conversion rate of the medical students compared with that in the general population again is manifest from the study. Thus, the former justly belong to the "special risk" group. The most recent findings of Karns[11] among the medical students at the University of Maryland bear out the increased incidence of conversions. The percentage of the positive skin test reactors among the medical students in the present study is significantly higher (14.4 per cent) than among the "general risk" group (5.9 per cent), closely approaches the total population (11.0 per cent), and is less than one-half of the rest of the "special risk" group (34.6 per cent).

The comparison of the data of the last two academic years reveals a slight increase in the positive reactions and in the conversion rates in 1959–1960 in both categories of students (table 2).

The proportion of converters and positive reactors did not change significantly between the "general risk" and the "special risk" groups during the two years. During this observation period, four active cases of pulmonary tuberculosis were detected. One of the active cases belonged to the "general risk" group and three of them to the "special risk" group.

It is believed that the above findings prove that proper grouping of a population may decrease the frequency of roentgenographic examinations and, consequently, the ionizing radiation hazard without increasing the hazard of the number of missed cases of tuberculosis.

* * *

The results of the study indicate that, in a university student group with an overwhelmingly young adult population, the tuberculin positivity and conversion rate is significantly different in the native white students from that in the foreign-born and Negro subjects. A special risk group exists which consists of the foreign-born, the Negro, and the medical students, with a significantly higher tuberculin sensitivity and conversion rate than the native white population. This justifies and indicates as practical and economical a tuberculosis control program different for the two groups. Such a program assures the adequate and more frequent control of the higher risk group. The method at the same time eliminates the unnecessarily frequent radiation hazard for the lower infection rate group. It is more convenient for the majority of

[11] KARNS, J. R.: Amer Rev Tuberc, 1959, *79*, 746.

the students, economical for the health service, effective for detection, and requires less time of personnel.

In view of the findings of this study, perhaps it will be possible to further reduce the frequency of microfilming. After the initial skin test and microfilm, repeat the skin test in the "special risk" group yearly, but use microfilms only in positive skin-test cases. Among the skin-test converters, substitute the regular size (14- by 17-inch) chest film for the microfilm. The present findings do not indicate more frequent skin testing and microfilming of the "general risk" group. The importance of close and adequate checkup and follow-up studies and search for the source of infection in every case of "conversion" should be emphasized as the best way to cope with the individual's problem and at the same time practice good preventive medicine.

In summary: In view of the hazards of tuberculosis and of radiation in respect to the tuberculosis control program, the student population of the University of Chicago was divided into a "general" and into a "special" risk group. The 5.9 per cent of the "general risk" and the 32.5 per cent of the "special risk" groups had positive skin test reactions. The conversion rate was 0.9 per cent for the "general risk" and 6.4 per cent for the "special risk" groups. Positive tuberculin skin test and conversion rate were statistically significantly higher among the foreign-born, the nonwhites, and the medical students than among the native white students. The findings indicate the necessity of more frequent skin testing and roentgenography in the "special risk" group, thus reducing the radiation hazard and the cost of the tuberculosis control program in the largest part (80.9 per cent) of university students without endangering the effectiveness of the program.

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August 1, 1960

THE ADEQUACY OF PRESENT CRITERIA OF NONINFECTIOUSNESS AS MEASURED BY TUBERCULIN CONVERSION IN THE OFFSPRING OF ADULTS WITH ACTIVE TUBERCULOSIS OF THE LUNG[1]

Based on the work of Riley and associates,[2] the assumption may be made that an "open" case of tuberculosis will infect a certain number of tuberculin-negative persons who remain in close contact with him over a period of months. "Open" refers to an individual from whose secretions human tubercle bacilli may be obtained, usually by cultural methods or guinea pig inoculation. If cultures and/or guinea pig tests are negative for tubercle bacilli, the individual is regarded as having a "closed" lesion.

The adequacy of this concept of "openness" or "closedness" has, so far as this writer can learn, never been clearly tested by a follow-up study of all tuberculin-negative children of patients who have received satisfactory medical discharges. Such a discharge is based on adequate therapy, roentgenograms, and what are regarded as sufficient bacteriologic examinations to establish noninfectiousness. The purpose of this study is to determine if negative sputum cultures truly reflect the discharged patient's status in relation to infectivity for a group of close contacts. Incidentally, such a study should also determine if the follow-up study of a group of tuberculin-negative offspring may allow, through the discovery of tuberculin conversion, the recognition of recurrent disease in the supposedly healed adult. In this light, the tuberculin-negative children of a household bear the same relation to a possibly infectious case as do the tuberculin-negative guinea pigs of the Wells-Riley experiment.

Families studied consisted of 51 groups who attend the Children's Tuberculosis Clinic of the Dallas Public Health Service. These families select themselves by the fact that the adult contact has at one time had active pulmonary tuberculosis and has achieved what is regarded as a satisfactory therapeutic result, including sputum conversion as measured by repeated cultures. The families must include tuberculin-negative children either as a result of the child's failure to convert prior to the parent's treatment or because of one or more live births subsequent to the parent's return to the family. The tuberculous parent must also have maintained a continuous contact with his offspring following his discharge. Finally, Mantoux tests of the children must be current.

All tuberculin-negative contacts are tested immediately and at six and twelve weeks following the parent's diagnosis. Upon return of the parent to the household, all tuberculin-negative children are tested at three, six, and twelve months, and thereafter annually. Test materials have consisted of either 5 TU of PPD, furnished by the U.S. Public Health Service, or 0.1 mg. of OT, furnished by the Texas State Department of Health. Some children have received as many as eight to ten Mantoux tests during the period of observation; a small number have received but a single test currently, after many years of contact. A few older children of these families have been observed only at the Tuberculosis Control Service, where study is limited to chest films. These are excluded from this study, as are children who have not reported back for tests since January, 1960. Also omitted from the study are families in which examination has not included all children. A breakdown of the results of Mantoux tests are presented in table 1.

The "primary date" is taken to be three months after institution of parental treatment, an interval during which all recently exposed children receive at least two Mantoux tests. Such a date should be used for all contacts who will have converted as a result of that particular exposure. The "secondary date" is used to designate, for tuberculin-negative children, the date of the parent's return to the household, or the date of birth of an infant following such return. The number of infants and children under study for each period of time subsequent to the resumption of exposure or "secondary date" is presented in table 2.

All but 8 of the source cases represent results of contemporary long-term antimicrobial therapy with isoniazid plus some other drug. Surgical measures have been applied in certain of the cases, mostly in the form of resection. Some of the adult patients have had one or more recurrences prior to their final attainment of a satisfactory result.

The adult patients for the most part have been treated in local Veterans Administration, Hospital District, or state hospitals. During much of the period these hospitals have required three negative cultures each month for three months prior to discharge. Subsequent cultures obtained progressively at three, six, and twelve-month intervals have been carried out by the Dallas Tuberculosis Control Division, Veterans Administration hospitals and Regional Office clinic, but the adults have frequently defected from their regular follow-up appointments.

In the case of three families for which the follow-up study has been more than six but less than

[1] This study was carried out under grant No. E-2063 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[2] Riley, R. L., *et al.*: Amer Rev Tuberc, 1957, *75*, 420.

TABLE 1

MANTOUX STATUS OF 231 CONTACTS OF INDEX CASES

Total children in 51 families		231	
Roentgenographic study only		19	
Mantoux-tested offspring			212
Born prior to primary Date	146		
Mantoux positive at primary date	55		
Mantoux negative at primary date		91	
Born subsequent to secondary date		67	
Mantoux negative offspring at risk			158
Converted	1		
Insufficient follow-up study	5		
Not retested since January, 1960	27		
	33		
Children in present study			125

TABLE 2

DURATION OF EXPOSURE FOLLOWING SECONDARY DATE

Contact Period	Number of Children
6–12 months	14
1–2 years	42
2–3 years	3
3–4 years	15
4–5 years	20
5–6 years	14
6–7 years	7
7–8 years	4
8–9 years	2
9–10 years	2
10 years	2
Total: 412 contact years	125

twelve months, the adult remains on drug regimens as an outpatient.

In the 51 families there are 231 offspring. Nineteen of these had roentgenographic examination only and, consequently, Mantoux tests were available on 212. At the primary date there were 146 offspring, of whom 55 (37 per cent) were Mantoux positive and 91 (63 per cent) were Mantoux negative. Born and surveyed since the secondary date are 67 children, one of whom converted and 6 of whom have not been tested since January, 1960. Of 91 who were negative at the primary date and who have subsequently been tested, 27 have not been retested within the past year, although their siblings have received tests. These 27 were negative at the time of the last Mantoux, however. (Most of these defectors are in late adolescence or early adult life and a conversion at their present age would not necessarily derive from the parental source.)

With all exclusions accomplished, there are 125 offspring of 51 tuberculous parents who have been followed for an average of 3.2 years of contact following the secondary date. One "conversion" has occurred. This took place in the six-year-old son of a female patient whose original disease dates from about 1940 and whose last clinical activity was in 1949–1950. Two children were born subsequent to that time and remained continuously negative till March, 1960, when the boy became tuberculin positive. The mother, however, had sought examination on account of persistent cough and had been discovered to have roentgenographic and bacteriologic relapse of her disease prior to the boy's visit for re-examination.

* * *

The low rate of relapse among adequately treated patients has been well documented.[3, 4] This group of 51 patients who had received satisfactory treatment and acceptable results resembles larger series in a relapse rate of 2 per cent.

But present concern is not with the relapse rate but with the "safety" of the culture-negative patient. If such an individual who is consistently negative on intermittent culture produced a few viable organisms during upper respiratory infections, would these be significant from the standpoint of infectivity? Are bacteriologic studies as currently a reliable index of the patient's infectivity?

The prolonged, intimate, and intensive contact of the "healed" parent with tuberculin-negative children, particularly in the unfavorable racial and economic conditions that characterize this group of families, should constitute a most rigorous test of the parent's infectiousness. It is apparent that the group of families is rather small. It is also true that some special factor may be operative in that only 37 per cent of the offspring were positive to tuberculin at the primary date. This percentage is lower than that for a much larger local group under study, but may be taken as near the average for the offspring of index cases excreting variable numbers of organisms.[5]

[3] WOROBEC, T.: Trans 18th Conf on Chemother Tuberc, Veterans Administration-Armed Forces, 1959, p. 173.

[4] LONGLEY, B. J., *et al.*: Trans 18th Conf on Chemother Tuberc, Veterans Administration-Armed Forces, 1959, p. 279.

[5] VAN ZWANENBERG, D.: Amer Rev Resp Dis, 1960, *82*, 31.

With all of these reservations, however, the significant finding is that only one child of 125 became tuberculin positive during a follow-up that averaged 3.2 years. This "conversion," as indicated above, occurred in the presence of obvious clinical, roentgenographic, and bacteriologic relapse in the parent, and very shortly following the relapse.

Since no conversions appeared in 124 children in whose parents the disease did not relapse, it seems permissible to infer that the present intermittent cultural method for tuberculosis is probably reliable and that the offspring of adequately treated index cases are at no greater risk than the average school child in the same social and economic group, whose conversion rate is about 2.5 per 1,000 per year.[6, 7]

In summary: During a 3.2-year follow-up study of 125 offspring of 51 supposedly healed adult index cases, one tuberculin conversion occurred in association with a frank relapse of the parent's disease. No conversions occurred in 123 offspring of 50 parents who remained well. Present cultural methods for tubercle bacilli seem to be adequate for the establishment of noninfectiousness.

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November 17, 1960

[6] CHAPMAN, J. S.: Minnesota Med, 1959, *42*, 1773.
[7] DALLAS TUBERCULOSIS ASSOCIATION: Unpublished data from school-testing program.

[8] The writer wishes to express his gratitude to Mrs. Margaret Dyerly, social worker, for her unflagging efforts to persuade families to return for follow-up study; and to members of the staff of the Dallas Tuberculosis Control Division; and to the staff of various hospitals who have cooperated most willingly in providing data. Special thanks are due to Mrs. Leona Hunter, R.N., of Dallas Tuberculosis Control Division for her cheerful and skilled assistance in the Children's Tuberculosis Clinic.

Correspondence

ENDOBRONCHIAL INSTILLATION OF ISONICOTINIC ACID HYDRAZIDE IN CHRONIC CAVITARY PULMONARY TUBERCULOSIS

To the Editor of the American Review of Respiratory Diseases:

We have attempted a new approach in the treatment of patients with long-standing extensive fibrocavitary pulmonary tuberculosis who could not or would not undergo surgery. The purpose was to bring the organisms into contact with high local concentrations of isoniazid. This follows Carabelli's[1] reported successes in the treatment of recent cases of pulmonary tuberculosis by the intrabronchial instillation of isoniazid suspended in oil into the segmental bronchus leading to the involved area.

Prior to bronchoscopy each patient had intensive sputum and roentgenographic studies, and these were continued at frequent intervals during the course of treatment. Also, susceptibility of the tubercle bacilli to various concentrations of isoniazid up to 1,500 γ per ml. was determined throughout the study.[2]

The suspension of 500 mg. of lyophilized isoniazid in olive oil was instilled through a polyethylene tube inserted into the lobar bronchus by bronchoscopy under topical anesthesia. Initially this was done at intervals of three weeks and later at biweekly intervals. Seven patients were treated with an average of 13 treatments per patient. All of these had old fibrocavitary pulmonary tuberculosis with infectious sputum.

The results of this study were disappointing. None of the patients showed any favorable roentgenographic changes or reversal of infectiousness. One patient, who initially did "convert" his sputum cultures to negative, turned up with two positive cultures again toward the end of his treatment. All patients stated that they felt better, in spite of no clinical evidence of this except that the bronchi of the involved lobe no longer showed injection of the mucosa and very little secretion after the third bronchoscopy in all patients. We did not encounter any unfavorable localized or systemic reactions to the procedure or to the drug.

We were fully aware that the acid-fast bacilli in some patients would show a high degree of resistance to isoniazid, but we thought that in most instances we might achieve a sufficiently high local concentration of the drug in and around the lesion with its acid-fast bacilli to overcome the resistance to the drug. This would have been in accord with Carabelli's assumption of a potential level of saturation of 842 γ per ml. in the local tissues. The bacteriologic status of the 7 patients is shown in table 1.

TABLE 1

Case Number	Susceptibility to Isoniazid	Catalase
1	Resistant at 1,500 γ	+
2	Resistant at 1,000 γ	+
3	Resistant at 100 γ	−
4	Susceptible	+
5	Susceptible	+
6	Susceptible	+
7	Susceptible	−

It is believed that, due to the marked fibrosis which was present in these patients as the result of their long-standing pulmonary infection, the bronchial lymphatics were occluded and, therefore, the drug could not be carried to the lesions. In addition, the fibrosis and scarring of the bronchial walls most likely prevented adequate adsorption of the material.

In summary, seven cases of fibrocavitary pulmonary tuberculosis were treated by the intrabronchial instillation of isoniazid without any favorable roentgenographic changes and without sputum "conversion." It is our opinion that this is not a satisfactory mode of treatment for this type of disease.

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November 3, 1960

[1] Carabelli, A. A.: Dis Chest, 1958, *34*, 162.
[2] Middlebrook, G.: Amer Rev Tuberc, 1954, *69*, 471.

Books

MEDICINE AND ANTHROPOLOGY. Lectures to the Laity, No. XXI, The New York Academy of Medicine. Edited by Iago Galdston. Pp. 165, International Universities Press, New York, 1959. Price, $3.00.

These six lectures were presented at The New York Academy of Medicine several years ago by a group of distinguished men from a variety of fields. Each looks to the relationship between medicine and anthropology from the vantage point of his own discipline—or, more properly, disciplines—for the greater number of the contributors are identified with more than one professional field. The selection of these men was especially fortunate, for they bring from their own backgrounds a breadth of interest and knowledge essential for drawing to a focus what until recently have been considered totally unrelated spheres of knowledge.

The success of the volume may be gauged by the fact that the reader is persuaded that these seemingly unrelated disciplines are indeed closely allied, at least in theory if not as yet in practice.

"The study of man," the broadest definition of anthropology, is of course a possible definition of medicine, with the added phrase "in sickness and health."

The message of this volume for the practicing physician, as well as for the medical researcher, is that the study of man as a biologic process cannot be completely understood without reference to the study of man as a social and cultural process. As this reviewer sees the relationship between these two sets of processes, they are intertwined and have been separated in the history of science for practical convenience.

But, as Marston Bates, an ecologist, F. S. C. Northrop, a philosopher, and John W. Dodds, a humanist, all point out, medicine must broaden its base to include the relevant aspects of cultural anthropology.

Man in his manifold relations to his environment—which, as a tool- and symbol-using animal he has remade—must increasingly become the subject for the physicians of the future. Epidemiology, and its application to public health practice, should build on a base of knowledge derived from studies of social and political organization as well as on modes of transmission of disease and population curves.

As Raymond Firth, the British anthropologist, has written (for this reader this is the single most outstanding essay in this volume), the acculturation process whereby one society takes over the culture of another, including its medicine, is relevant. It may be crucial to the successful extension of medicine to peoples in the economically underdeveloped areas of the world where curing, both in etiology and practice, are based on tribal and folk traditions.

Dr. Paul Fejos, a physician turned anthropologist, elucidates the concepts of magic, so important in primitive medicine and essential for the full understanding of the psychology of peoples in the primitive world who are now in contact with scientific medicine.

Dodds, as an educator in the humanities, points out the pressing need for liberalizing the medical curriculum, but he fails to suggest how this can be brought about practically in the face of vested interests within the divisions of medical schools.

Dr. Alexander H. Leighton, as a social psychiatrist, has recognized and done valuable research on the tremendous importance of the rate of culture change in its effect on the mental health of a population.

This book points up the ideologic bases for closer integration of these two vast fields of knowledge. The thoughtful physician will not disagree with the broad argument, so well stated by Dr. Galdston in his Introduction, but he may find fault with the lack of any suggested plan in the essays which follow as to how such integration can be brought about in terms of the education of physicians. The task facing the medical educators and the leaders in anthropology is tremendous, for it calls for planned change in professional fields, each of which has its own fixed interests that are not easy to overcome. Successful collaborative research may lead the way and set the example for the educators.

The conservative anthropologist will raise the question: Anthropology may serve to advance medical knowledge, but can such application advance anthropologic theory? This is not just carping criticism, for effective collaboration must be enriching to *both* fields.

To this the reviewer rejoins: Theories of culture change need to be put to the test, not only in comparative studies of natural change, but in long-range studies of directed change. Medicine, especially in the fields of public and mental health, offers such proving grounds par excellence.

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HANDBUCH DER TUBERKULOSE. Volume I: Allgemeine Grundlagen. Edited by J. Hein, H. Kleinschmidt, and E. Uehlinger. Pp. 832, Georg Thieme Verlag, Stuttgart, 1958. Price, DM 178 per volume singly; DM 142.40 per volume for set of five.

The book under review continues the great tradition of the German medical handbooks, compiled and edited for each specialty, which won a well-deserved international reputation in the early part of this century. How much can these reference works contribute to the training of the modern, scientifically oriented clinical specialist? The reviewer remembers distinctly a remark made two years ago by the eminent German virologist, Prof. Henneberg, that the "era of the handbooks" is dead and that the future belonged to the carefully compiled bibliography, accompanied by a brief, factual, impartial text (a method which was, for instance, followed in Prof. Raettig's recent work on bacteriophages). Even if this is true—and further developments might very well go in this direction—one is compelled to admit that the handbook under review fails to show signs of decay or impending death.

An endeavor on so grand a scale as the present work must by necessity indicate a historical review of the tuberculosis problem, and this task has been brilliantly discharged by Prof. Löffler of Zürich, the internationally known internist. In short, concise chapters, frequently using easily readable tables, Prof. Löffler unveils before the reader the seemingly endless road of trial and error which mankind had to transgress in its search for the cause of tuberculosis. The recognition of the infective character of the disease and the intelligent hygienic and dietary measures conceived by the Greeks and Romans fell into oblivion, eventually to be revived again in the early Rennaissance. The hygienic measures, carried to excess by the public health laws of Philip IV of Naples, actually delayed the final victory which was won—over countless last minute errors and setbacks (among these the failure to recognize the tuberculous character of scrofulosis by Rudolf Virchow and some eminent French clinicians—in 1882 by Koch. The reader will find it difficult to tear himself away from this fascinating chapter before finishing it, not least because of the sections devoted to the development of the sanatorium movement and to the discovery of the antimicrobials.

Professors Suter (Gainesville) and Bloch (Pittsburgh), neither of whom needs any introduction to the reader in the United States or Europe, have contributed two brilliant chapters on the biology of the tubercle bacillus and the immunobiology of tuberculosis. Reviewing the literature on nonspecific factors influencing experimental tuberculosis, they stress the vital role which host resistance plays in determining the virulence of the tubercle bacillus. For this reason, difficulties arise at once if the investigator attempts to determine the LD_{50} for a given strain which will largely depend on the stability of the latter and the mode of infection. All important media currently used for the cultivation of the tubercle bacillus are critically reviewed and the reliability of the counting methods is discussed at length, whereby particular emphasis is laid on Fenner's dilution procedure. A comprehensive report on the metabolism of the tubercle bacillus follows in which the authors justly stress the necessity to re-examine the data known at present by studying the behavior of the tubercle bacillus in the tissues of the host. The reader interested in morphology will find an excellent compilation of the pertinent information, as well as detailed data on the chemistry of the bacillus and a review of the numerous extraction methods employed to investigate its chemical structure (an excellent table will be found on page 164). From this, Drs. Suter and Bloch logically develop their views on the antigenicity of the various fractions of the tubercle bacillus and the specificity of tuberculin and PPD, the potentialities of living and killed vaccines, and the role of the allergic reaction in the mode of spread of the tubercle bacillus.

The last mentioned question, i. e., the problem of the significance of altered reactivity in the spread of a tuberculous infection, is taken up in great detail in the following three chapters. Professor Fassbender of Mainz introduces his contribution with a careful definition of the subject, and points out that the term allergy has undergone considerable changes from the times of Clemens von Pirquet, who coined it in 1908, to Robert Rössle, who gave it its present meaning in 1932 by subdividing it into the three reaction forms of hyperergy, hypergy, and anergy. Professor Fassbender stresses that allergy can always only be one of many factors determining the outcome of the tuberculous infection. He then gives an impartial account of the two opposing views of Hübschmann and Uehlinger on the relations between reaction form and anatomic picture in the infected host, both of which are too complicated to be abstracted here. Greater agreement is present as to the significance of the epithelioid cell which is conceived to be the landmark of a successful host response to the antigen. The hyperergic state is accompanied by hematogenous spread, cavity formation indicates a labile state, and the acinar-nodular type of phthisis of the German literature indicates an anergic state. The chapter is concluded with a brief discussion of the role of allergy in the pathogenesis of sarcoid. The

clinical implications of altered reactivity are then discussed by Prof. Schwengler with reference to exudative pleurisy, asthma (the possibility of a specific tuberculous asthma is suggested), myocarditis, mesenchymal hepatic reaction, interstitial nephritis, erythema nodosum, and tuberculous polyarthritis. The latter includes a most interesting re-evaluation of the observations of Poncet and Berger. The author does draw attention to the possibility of a simultaneous sensitization against streptococci, a point which certainly needs further consideration.

The internationally famous Prof. Ferdinand Schmid, now living in "active retirement" at Heidelberg, gives in his contribution a fascinating analysis of the various factors influencing host resistance, such as race, species, constitution (he differentiates a hyper- from a hypomesenchymatous type), and age. The reader is referred to the very interesting tables on pages 296 and 297. Particular emphasis is placed on the importance of the monocyte and those factors which depress resistance, among which he includes superimposed infection, radiation, and metabolic disease, as well as local factors. The authors share Rich's views on the differences between allergy and immunity.

Professor Kleinschmidt, one of the co-editors of the Handbook and professor emeritus of pediatrics at the University of Göttingen, has devoted his contribution to the questions of tuberculin and tuberculin sensitivity. He points out that its most important characteristics are the absence of antigenicity and the slow toxicity which produces death in the guinea pig only after repeated injections of high doses. There are two principal methods to produce tuberculin, first by fractionation of the whole bacillus at low temperatures, and second by using the heated culture filtrate. The various ways of transferring or suppressing tuberculin hypersensitivity are discussed. This also includes an interesting analysis of the phenomenon of parallergy which is occasionally observed after smallpox vaccination. After a detailed review of the literature the author concludes that at least 100 international units must be injected intracutaneously in order to exclude or prove tuberculosis.

Dr. Herrmann, an outstanding public health officer, has contributed a chapter on the serodiagnosis of tuberculosis, a field which has recently again evoked widespread interest after many years of near oblivion. Dr. Herrmann, who discovered the bacillary antigen "Essen," compares its specificity with those described by other authors, such as the antigens "Bonn," "Japan," and "Beller." To date, even the most reliable antigens give a positive reaction in only 80 per cent of cases of tuberculous meningitis.

Professor Redecker, president emeritus of the Federal Department of Health of West Germany, does not require any introduction to the English-speaking chest specialist. His article on the epidemiology of tuberculosis undoubtedly belongs among the most outstanding in the Handbook. Beginning with a short historical essay, he formulates argument after argument to demonstrate the close association between the advancement of civilization and the progress of tuberculosis. If the disease breaks into an area heretofore untouched, a mortality curve with an adolescent and an old-age peak will result. With further "Durchseuchung," the second peak will flatten out. It is postulated that the higher mortality curve of males fuses with the generally lower female mortality once social legislation has removed the worst excesses of professional and social competition from men. In making this point, the author advances a wealth of statistical material. (Unfortunately, the last American statistics used date back to 1934.) The close relationships between mortality and starvation are reflected in figures accumulated during the two world wars as, for instance, in the British mortality rate which started to decline almost at once after the worst threat of the German submarine blockade had been overcome in 1942.

Sources of tuberculous infection and mode of spread are reviewed by Prof. Hedvall, the internationally known head of the central dispensary at Uppsala. The reader will find here an immensely fascinating survey of the recent literature and an extract of the author's vast experience. Professor Hedvall very justly demands closer familiarity with the "forgotten" sources of infection, namely, water and sewage. Tonsils and bronchi are stressed as sites of entry, and a comprehensive account is given of P. H. Schwartz's theory of the bronchial penetration.

To the great credit of the editors of the Handbook, the article on "determinants of spread" has been entrusted to the outstanding geneticist, Prof. Diehl, who won international recognition through his large-scale investigtaions on pure-bred rabbits and his observations on twins (a large number of which were pursued in association with Von Verschuer). The enormous amount of accumulated material prohibits its detailed discussion in this review, but it may be stated that the reviewer knows of no other present source from which the interested reader might obtain a similarly comprehensive survey of the subject. Professor Diehl considers in great detail studies on twins conducted both in the United States and on the continent by Kallmann/Reisner, Planansky, Alten, Diehl/Verschuer, et cetera, and then gives

a brilliant analysis of his own animal studies and those of Lurie, Wright and Lewis, Küster and Kronig, and G. Weber. He points out that the greatest caution is indicated in the interpretation of their often contradictory results, but he believes that the following conclusions are justified: (*1*) Pulmonary and peripheral tuberculosis are to a certain extent mutually exclusive. (*2*) An hereditary trait to develop peripheral manifestations of tuberculosis exists in man. (*3*) Twins have a propensity to manifest similar forms of late, generalized tuberculosis. In man, a hyper-reactive–lymphatic reaction occurs which shows a good tendency to deal successfully with the infection.

The well-known Swiss chest specialist, Prof. Ott, has undertaken in two chapters the eminently difficult task of reviewing environmental, age, and sex factors influencing infection with and spread of tuberculosis. He points out the fallacy of making shortage of food alone responsible for an outbreak of the disease, using a particularly well-documented example. Tuberculosis mortality rose in Holland almost at once after the defeat of the country at a time when the rationing system still provided approximately 2,300 calories per day for the average consumer, while, in Denmark, where the consumer was able to obtain approximately 3,000 calories per day, tuberculosis mortality continued its gradual prewar downward trend. The situation in unoccupied Switzerland was similar to that in Denmark, although the daily caloric intake was somewhat less. When, however, actual famine began in Holland in late 1944, the death rate for tuberculosis started to climb out of proportion to its previous trend, now demonstrating a clear association to lack of supplies. Ott follows an analysis of similar observations by Gorrstein in Finland and Wolff in Germany which demonstrates a retardation of growth still evident for several decades among the members of the generation born during the hunger period. Consequently, the highly important observations are quoted which were made by Giese on autopsy material during and after World War II, which revealed in starving or undernourished tuberculous patients forms of spread hitherto unobserved. Professor Ott reviews the available evidence for the influence of age on the disease, and concludes that infants generally show a greater ability to deal successfully with the infection than do babies, and that the age group of greatest risk comprises more young adults who are about to begin their professional careers if, otherwise, the general "Durchseuchung" is low.

The last chapter, written by Prof. Cohrs, is devoted to the epidemiology and pathogenesis of veterinary tuberculosis. At the time of publication, 38.5 per cent of the cattle in Germany were still infected with tuberculosis, but the government plans to eradicate the disease completely in the next ten years. The importance of the goat and the dog as carriers is stressed, and a detailed account is given of the course of tuberculosis in cattle.

While the publication of the remaining four volumes of the Handbook must be awaited, it should be stated now that the work in its present form represents an extraordinarily well-planned encyclopedia of our knowledge of tuberculosis. Acquisition of this volume is recommended to all scientifically oriented workers in this field.

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BOOKS RECEIVED

CLINICAL CARDIOPULMONARY PHYSIOLOGY. Second Edition. Edited by Burgess L. Gordon *et al.* Pp. 1001, Grune & Stratton, Inc., New York, 1960. Price, $28.50.

AN OUTLINE OF CHEMICAL GENETICS. By Bernard S. Strauss. Pp. 188, W. B. Saunders Company, Philadelphia, 1960. Price, $5.00.

FUNDAMENTALS AND POSSIBILITIES IN ANTI-TUBERCULOSIS VACCINATION. By Richard Prigge and Günther Heymann. Pp. 108, University of Toronto Press, Toronto, Canada, 1960. Price, $5.00.

A SYSTEM OF MEDICAL HYPNOSIS. By Ainslie Meares. Pp. 484, W. B. Saunders Company, Philadelphia, 1960. Price, $10.00.

PAGES IN THE HISTORY OF CHEST SURGERY. By Rudolf Nissen and Roger H. L. Wilson. Pp. 166, Charles C Thomas, Springfield, Illinois, 1960. Price, $7.50.

YEAR BOOK OF GENERAL SURGERY (1960–61 Series). Edited by Michael S. DeBakey. Pp. 616, Year Book Publishers, Inc., Chicago, 1960. Price, $8.00.

SYMPOSIUM ON GUANETHIDINE (Ismelin®). The University of Tennessee College of Medicine. Pp. 119, Ciba Pharmaceutical Products, Inc., Summit, New Jersey, 1960.

American Thoracic Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21–25
1962—Miami Beach, Florida—May 20–24
1963—Denver, Colorado—May 12–16
1964—New York, New York—May 24–28

CHEST PHYSICIANS ESTABLISH FUND TO ASSIST CUBAN MEMBERS

At the meeting of the Board of Regents of the American College of Chest Physicians held in Washington, D. C., on November 28, 1960, a resolution was adopted to establish a relief fund for Cuban members of the College who have been exiled temporarily from their country. The Board of Regents voted to contribute $5,000 to launch the fund, and contributions are being solicited from College members and others who are interested. The Cuban Chapter of the College was founded in 1940 and now has 74 members.

For further information, contact Murray Kornfeld, Executive Director, American College of Chest Physicians, 112 East Chestnut Street, Chicago 11, Illinois.

ANNOUNCEMENT

TRUDEAU SCHOOL OF TUBERCULOSIS AND OTHER PULMONARY DISEASES

FORTY-SIXTH SESSION, 1961

The Trudeau School of Tuberculosis and Other Pulmonary Diseases, which will hold its forty-sixth session in Saranac Lake, New York, from June 5 to 23, 1961, continues to provide a unique opportunity for training in the field of chest diseases. This annual postgraduate course for physicians, conducted under the auspices of the Trudeau Foundation and supported by the Hyde Foundation, is able to provide outstanding instruction at a minimal tuition of $100.00 for a three-week session. Attendance at the Trudeau School carries with it a thorough review for specialization in pulmonary disease or for work in public health involving tuberculosis.

In addition to the local medical faculty consisting of some thirty doctors from Saranac Lake, Ray Brook State Tuberculosis Hospital, and the Sunmount Veterans Administration Hospital, about thirty of the leading teachers and investigators in the Eastern United States and Canada are brought to Saranac Lake each year to lecture or to conduct seminars in their special fields. Approximately half of the time is devoted to tuberculosis, and the other half divided between such subjects as silicosis, pulmonary fibrosis, emphysema, fungous infection, sarcoidosis, pneumonias, and intrathoracic tumors.

The enrollment is necessarily limited and, therefore, application should be made early. A few scholarships are available for those who qualify.

Inquiries should be addressed to the Secretary, Trudeau School of Tuberculosis and Other Pulmonary Diseases, Box 670, Saranac Lake, New York.

INTERNATIONAL UNION AGAINST TUBERCULOSIS

XVIth INTERNATIONAL TUBERCULOSIS CONFERENCE

The XVIth International Tuberculosis Conference, under the presidency of Dr. G. J. Wherrett, will be held in Toronto, Canada, September 10–14, 1961, delegates from sixty-eight member countries of the Union attending.

An international committee has arranged a comprehensive program covering many aspects of current thought on tuberculosis, and there will be presentations on the latest work from many countries. The participation of distinguished speakers from all over the world will make this an outstanding international conference. A special feature will be the simultaneous interpretation of the various sessions into English, French, German, Russian, and Spanish.

Toronto has excellent facilities for such a conference. All of the meetings will be held at the Royal York Hotel, and there will be ample accommodation there and at other nearby hotels.

Administrative sessions of the Union will be held in Toronto from September 7 to 10. Meetings of technical committees are expected to be held on September 8, 9, and 10. These committees are concerned with health education, radiology and mass roentgenography, surgery of tuberculosis and collapse therapy, epidemiology, laboratory methods, domiciliary and ambulatory treatment, BCG, antimicrobials and chemotherapy, and occupational therapy and rehabilitation.

On Sunday, September 10, the inaugural session will be held, at which the distinguished patrons of the Conference, headed by the Governor General of Canada, the Minister of National Health and Welfare, and the Minister of Health for the Province of Ontario, are expected to be present.

The outline of the scientific program is:

Monday, September 11:

9 A.M.–1 P.M.: Primary drug resistance of the tubercle bacillus (bacteriologic, therapeutic, and epidemiologic aspects of the problem).
Chairman of the panel: Prof. B. Kreis (France); Principal rapporteur: Dr. G. Meissner (Germany).

2.30–5.30 P.M.: Primary drug resistance of the tubercle bacillus (bacteriologic, therapeutic, and epidemiologic aspects of the problem). Discussion.

2.30–5.30 P.M.: How to inform the general public that tuberculosis is not yet under control.
Chairman of the panel: Dr. G. J. Wherrett (Canada); Principal rapporteur: Dr. Margaret E. Nix (Canada).

2.30–5.30 P.M.: Free communications.

Tuesday, September 12:

9 A.M.–1 P.M.: Pulmonary tuberculosis in persons older than fifty years of age (epidemiologic, clinical, and therapeutic aspects).
Chairman of the panel: Prof. A. Omodei Zorini (Italy); Principal rapporteur: Prof. Ph. V. Chebanov (U.S.S.R.).

2.30–5.30 P.M.: Pulmonary tuberculosis in persons older than fifty years of age (epidemiologic, clinical, and therapeutic aspects). Discussion.

9 A.M.–1 P.M.: Prospective role of voluntary tuberculosis associations throughout the world.
Chairman of the panel: Dr. T. Gedde-Dahl (Norway); Principal rapporteur: Dr. J. Meijer (The Netherlands).

2.30–5.30 P.M.: Methods of improving and checking the taking of prescribed drugs by tuberculous patients (study of the problem in consideration of the different sociologic and economic conditions prevailing).
Chairman of the panel: Dr. P. V. Benjamin (India); Principal rapporteur: Dr. Wallace Fox (Great Britain).

2.30–5.30 P.M.: Free communications.

Wednesday, September 13:

9 A.M.–1 P.M.: New concepts regarding the significance of tuberculin sensitivity (with special reference to the problem of low-degree sensitivity).
Chairman of the panel: Dr. Nissen Meyer (Norway); Principal rapporteurs: Dr. P. D'Arcy Hart (Great Britain), Dr. Carroll E. Palmer (U.S.A.).

9 A.M.–1 P.M.: Respiratory insufficiency in patients with pulmonary tuberculosis.
Chairman of the panel: Prof. J. K. Kraan (The Netherlands); Principal rapporteur: Dr. P. Sadoul (France).

2.30–5.30 P.M.: The problem of rapid inactivation of isoniazid.
Chairman of the panel: Dr. A. R. Armstrong (Canada); Principal rapporteur: Dr. L. H. Schmidt (U.S.A.).

2.30–5.30 P.M.: The problem of the relation between sarcoidosis and tuberculosis.
Chairman of the panel: Dr. Sven Löfgren

(Sweden); Principal rapporteur: Dr. J. G. Scadding (Great Britain).

2.30–5.30 P.M.: Free communications.

Thursday, September 14:

9 A.M.–12.30 P.M.; 2 P.M.–4 P.M.: The eradication of tuberculosis in different countries according to existing conditions (theoretical problems and practical solutions).

Chairman of the panel: Prof. Walsh McDermott (U.S.A.); Principal rapporteur: Dr. G. Canetti (France).

Each major session of the Conference will be presided over by a chairman. A principal rapporteur will cover the subject in broad outlines, particular aspects then being presented in detail by five panel members. There will then be ample opportunity for discussion from the floor and, for this purpose, three additional half days have been set aside in the afternoons for continuation of discussion on the subjects presented at morning sessions. There will also be three sessions for "free communications." At these sessions, original papers may be presented on any subject concerned with tuberculosis.

Speakers who wish to take part in the discussions on major topics of the Conference, or to present "free communications," are required to submit their names and a summary of their remarks to the program committee beforehand so that a selection may be made: the closing date for receipt of these summaries is May 1, 1961. Those from the United States who wish to make contributions should inform the Executive Secretary, National Tuberculosis Association, 1790 Broadway, New York 19, New York. Those from Canada and South America should inform the Secretary, XVIth International Tuberculosis Conference, 265 Elgin Street, Ottawa, Canada.

There will be a large scientific, technical, and commercial exhibition in connection with the Conference. Visits to hospitals in the city of Toronto are planned. Delegates will be given an opportunity to see the latest in the Canadian medical world through visits to Western Hospital, Sunnybrook Hospital, Toronto Hospital for Tuberculosis, The Charles H. Best Institute, and the Hospital for Sick Children.

A full program of receptions and social events is being planned for all who attend. These will include the Presidential banquet, receptions by the Government of Ontario and the City of Toronto, an orchestral concert, a boat trip on Lake Ontario, and visits to art galleries, museums, and theaters. An all-day tour of Niagara Falls is being arranged, and there will be a special ladies' program on each day. At the conclusion of the scientific sessions, there will be a postconference tour of the St. Lawrence Seaway, Ottawa, Montreal, and Quebec City and, in cooperation with the National Tuberculosis Association, visits in New York City to the Health Department, Bellevue Hospital, the Rockefeller Institute, and a special reception at the United Nations.

The registration fee for the Conference will be $10.00 per person. Full details regarding the program, registration, hotel accommodation, social events, and tours may be obtained from the Secretary, XVIth International Tuberculosis Conference, 265 Elgin Street, Ottawa, Canada.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE
Editorial Assistant: TERRI MANN

VOLUME 83 | March 1961 | ABST. No. 3

The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 42 abstracters systematically review 239 journals which represent 36 countries and which are published in 22 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk(*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Bronchial Obstruction Secondary to an Aberrant Pulmonary Artery. J. R. Derrick* and H. Stoeckle. *AMA J Dis Child*, June, 1960, 99: 830-832. (*U. of Texas Medical Branch, Galveston)

A congenital aortic ring was the suspected cause of respiratory obstruction in an infant with the presenting symptoms of stridor, dyspnea, cyanosis, and a barking cough. All antemortem studies yielded negative results, but postmortem examination revealed an aberrant left pulmonary artery which compressed the right main bronchus. Bronchoscopy, which has been the most useful diagnostic tool in other instances of this condition, was not performed in this case. This report was published to emphasize that even repeated iodized oil or barium contrast visualization of the esophagus sometimes misses extrinsic pressure from aberrant vessels, resulting in incorrect diagnosis.—M. J. Small

The Solid Sarcomatous Pulmonary Artery. J. E. Jacques* and R. Barclay. *Brit J Dis Chest*, July, 1960, 54: 217-220. (*London Chest Hosp., England)

Two patients are reported with the finding of a solid pulmonary artery at thoracotomy. In both, the preoperative diagnosis was primary pulmonary neoplasm, and the ultimate histologic diagnosis was sarcoma occupying the whole lumen of the main pulmonary artery with extension along its branches. It is believed possible to make a correct preoperative diagnosis in this uncommon condition. In both cases presented, the lack of peripheral vascular markings on the affected side was quite obvious. The hilar vascular markings, on the other hand, were of increased density. This should have been noted and could have been a major factor in arriving at a diagnosis. One case was resectable by pneumonectomy. The second case was found on angiocardiography to have a filling defect in the right ventricle. It is suggested that this was the primary tumor, metastasizing to the pulmonary artery. It is also possible that in some of the other 8 previously reported cases a similar etiology prevailed.—M. J. Small

Electrocardiogram in Asthmatic Children. T. Miyamoto*, I. Kass, and M. S. Hoffman. *Circulation*, July, 1960, 22: 90-95. (*National Jewish Hosp., Denver, Colo.)

Electrocardiograms of 137 asthmatic children 5 to 16 years of age were compared with findings in normal children. The following deviations from the norm were found: lower voltage and longer duration of P waves in all leads; lower voltage of R waves in all precordial leads and almost all limb and unipolar leads; deeper S waves over left precordial leads; slight delay of intrinsicoid deflection in right but not in left precordial leads; somewhat increased duration of QRS complexes; increased heart rate in the older age groups, but no differences in P-R and Q-T intervals; and predominantly vertical electrocardiographic position of the heart. These observations provide further electrocardiographic evidence that asthmatic children show a tendency toward right ventricular hypertrophy and hyperinflated lungs.
—W. J. Steininger

Effect of Smoking on Asthma and Emphysema. G. A. Peters* and R. D. Miller. *Proc Mayo Clin*, June 22, 1960, 35: 353-358. (*Mayo Clinic, Rochester, Minn.)

The literature is reviewed on the association between smoking, bronchitis, asthma, and obstructive emphysema, and 2 cases are reported of patients with pulmonary insufficiency whose function studies showed marked improvement after complete cessation of smoking.—E. A. Riley

Asthmatic Symptoms in the First Year of Life. P. Selander*. *Acta Paediat (Upps)*, May, 1960, 49: 265-269. (*Flensburg Children's Hosp., Malmo, Sweden)

Of 1,092 children with asthmatic symptoms who were treated during the period from 1949 to 1958 at the Flensburg Children's Hospital, 47 per cent had developed their first symptoms between the ages of 0 to 1 year. In 87 per cent the symptoms had appeared before 5 years of age. Boys comprised 63 per cent of the cases. From age 7, the risk of becoming ill was equal for boys and girls. Eighty per cent of those who had had asthmatic symptoms in infancy were symptom-free at the age of 6 to 11 years. An acute infection can be demonstrated in the majority of infants with asthmatic symptoms. The season of the first occurrence of the asthmatic symptoms coincided with that of acute upper respiratory infections. Children who had asthmatic symptoms in infancy, and who at the time of the investigation were 6 to 11 years old, formed a group between children of the same age with genuine bronchial asthma and a control group with regard to heredity to asthma and eczema and the risk of developing eczema and hay fever. They also ran an increased risk of developing genuine bronchial asthma but not of developing upper respiratory infections (after author's summary).—H. J. Simon

Cardiac Injury Due to Nonpenetrating Chest Trauma. J. WATSON* and W. BARTHOLOMAE. *Ann Intern Med*, April, 1960, 52: 871–880. (*Akron General Hosp., Ohio)

Forty-four patients with nonpenetrating chest injuries were observed for evidence of cardiac trauma. This study was designed to compile accurate statistics as to the frequency of cardiac injury in a consecutive series of chest injuries. The figures at this time indicate an incidence of approximately 38 per cent. The average duration of electrocardiographic abnormalities was 15 days, with a range of from 4 to 30 days. No permanent electrocardiographic changes were found which could definitely be attributed to trauma. Only 1 had any clinical manifestations of cardiac trauma. An attempt to correlate transaminase levels with electrocardiographic changes as diagnostic procedures proved fruitless. To study chest injuries thoroughly, an electrocardiogram should be included to determine even minor amounts of myocardial damage. Prompt recognition of the presence of cardiac trauma is necessary in order that significant, often fatal, manifestations may not be overlooked.—T. H. NOEHREN

Isolated Cryptococcosis Associated with Boeck's Sarcoid. Report of a Case Treated with Amphotericin B. L. A. BERNARD* and J. C. OWENS. *AMA Arch Intern Med*, July, 1960, 106: 101–111. (*Hermann Hosp., Houston, Texas)

A case of coexisting sarcoidosis and localized cryptococcosis, the seventh recorded in the literature, is presented. This 44-year-old Negro housewife had pre-existing sarcoidosis of 3 years' duration when isolated vertebral and pulmonary cryptococcal lesions were discovered. Good response occurred after therapy with amphotericin B. The pathologic and clinical pictures produced by sarcoidosis and cryptococcosis are surprisingly similar. Indeed, when the organism *C. neoformans* cannot be identified, as is often the case in tissue sections, it becomes impossible at times to distinguish between these 2 conditions. Sarcoid disease alone seldom, if ever, causes suppuration and abscess formation, however. Clinically, both diseases may produce pulmonary symptoms, generalized lymphadenopathy, hepatosplenomegaly, and bone involvement. Involvement of the meninges is more frequent in cryptococcal infections. It is suggested that the presence of "benign" or asymptomatic cryptococcal infection is frequently overlooked.—W. J. STEININGER

Bronchial and Pulmonary Carcinoid, and Bronchial and Pulmonary Microcarcinoidosis (in German). F. FEYRTER. *Wien Klin Wschr*, June 3, 1960, 72: 386–392.

Bronchial and pulmonary carcinoids develop from the endocrine argyrophil H-cell organs of the bronchial tree, the former from the larger bronchi, and the latter from the bronchioles and respiratory bronchioles. Bronchial carcinoids produce 5-hydroxy-tryptamine (serotonin), causing the carcinoid syndrome in malignant bronchial carcinoid. Compared to this "grand mal," benign bronchial carcinoid most probably causes symptoms of a "petit mal." Bronchial and pulmonary microcarcinoidoses originate from a disseminated hypertrophy of the argyrophil bronchial H-cell organ. —G. C. LEINER

Cushing's Syndrome and Bronchogenic Carcinoma. J. LEWIS*, N. SEARLE, and G. JORDAN. *Ann Intern Med*, May, 1960, 52: 1138–1146. (*Baylor U. College of Med., Houston, Texas)

A patient is presented who had Cushing's syndrome and bronchogenic carcinoma of the reserve-cell variety. A comparison of the clinical features in this patient with those observed in 7 other reported cases in which these 2 processes existed indicates a marked similarity. It seems unlikely on the basis of chance alone that 8 such similar cases should have occurred unless there is some direct relationship between the 2 diseases; however, at the present time there is no objective evidence to support this concept (after authors' summary). —T. H. NOEHREN

Morphology of Carcinoma of the Lung, with Special Reference to Its Histologic Classification (in Japanese). T. MIYAJI*, S. TADA, H. SANDO, and G. RYUFU. *Jap J Chest Dis*, June, 1960, 19: 381–390. (*Osaka U. School of Med., Japan)

The writers reviewed 951 cases of lung cancer which were confirmed at autopsy during the past 10 years at various institutions in Japan. The maximal number of cases were seen in the 6th decade, especially in the age group of 50 to 54 years. The tumor was seen more frequently in the right lung, and in the upper lobes of both lungs. The tumor was macroscopically divided into 8 types—lumpy, infiltrative, cavitary, disseminated, hilar, mediastinal, pleural, and bronchiolar—but the lumpy type was most frequently seen (in 373 of 704 cases). The criteria of the histologic classification of lung cancer are described and divided into 3 types: adenocarcinoma, squamous cell, and undifferentiated carcinomas. Adenocarcinoma was seen most frequently in the 738 cases examined histologically, and appeared to be peculiar to this illness in the Japanese, although the high incidence of adenocarcinoma was common to Japanese, European, and American women. The authors stress that adenocarcinoma, especially the

less differentiated adenocarcinomas, were liable to be classified as other forms of lung cancer. The histologic types had no relation to age, but had some relation to the length of survival.—I. Tateno

Clinical Experiences with Intravenous Colchicine in Inoperable Bronchogenic Carcinoma. S. Cohen* and J. Johnson. *Dis Chest*, July, 1960, 38: 30–41. (*Pollak Hosp. for Chest Diseases, Jersey City, N. J.)

Colchicine has long been known to have cytotoxic, antimitotic, and anti-tumor properties, but has received little attention in its application to chemotherapy in human cancer. The availability of an intravenous preparation prompted the present clinical study. Ten patients with advanced bronchogenic carcinoma were treated with colchicine, and an equal number with colchicine combined with conventional irradiation. Large amounts of colchicine (2 to 4 mg.) administered intravenously were well tolerated. The colchicine–X-ray–treated group seemingly fared better.—E. A. Rouff

Cytomegaly in the Adult (in German). H. Chiari*. *Wien Klin Wschr*, June 3, 1960, 72: 382–386. (*Pathologisch-Anatomisches Institut der Universität Wien, Austria)

A 38-year-old woman had pneumonia which did not respond to antimicrobials or steroids. She died from an infectious hepatitis. At autopsy, peribronchial pneumonic infiltrations were found. Microscopic examination revealed the presence of giant cells measuring 30 to 40 μ which stained like cytomegalic cells; their nuclei appeared like "owls' eyes," described as characteristic for the presence of inclusion bodies. In addition, moniliasis of the lungs was found. The literature on cytomegaly, a virus infection rare in adults and more frequent in children, is reviewed. The lungs are involved more often than any other organ. It is possible that in this case the cytomegaly of the lung was an accidental finding.—G. C. Leiner

Progressive Pulmonary Dystrophy (in German). P. Kröker*. *Fortschr Roentgenstr Nuklearmed*, July, 1960, 93: 1–20. (*Evangel. Krankenhaus, Huyssens-Essens, Germany)

So-called progressive pulmonary dystrophy is characterized roentgenographically by increased pulmonary translucency. It may be caused by: (*1*) "vanishing lung," which is due to bullous emphysema, (*2*) the cystic lung picture with its decrease in vascularity, and (*3*) pulmonary hypoplasia or aplasia with the resulting replacement of pulmonary vessels by bronchial vessels. The latter two conditions rarely cause symptoms. An unusual cause of progressive pulmonary dystrophy is a thrombosis of the main pulmonary artery. —H. Abeles

Tracheotomy for Acute Pulmonary Insufficiency Complicating Chronic Pulmonary Emphysema. L. B. Tecimer and M. L. Pearce*. *AMA Arch Intern Med*, June, 1960, 105: 891–898. (*V.A. Center, Los Angeles, Calif.)

The value of tracheotomy in the management of severe episodes of acute pulmonary insufficiency has received little attention in medical literature. Four illustrative cases, chosen because adequate physiologic data were available, are presented to emphasize the importance of early tracheotomy in the management of these cases. Tracheotomy has proved to be of value in the treatment of progressive and intractable respiratory insufficiency in patients with chronic pulmonary emphysema by permitting frequent aspiration of secretions. It is a minor operation which should be considered in any patient with respiratory insufficiency who is deteriorating in spite of conservative treatment. The procedure is mandatory if a respirator is to be used. Complications have been uncommon in the authors' experience.—W. J. Steininger

Some Observations on Acute Bronchiolitis in Infants. S. H. W. Sell*. *AMA J Dis Child*, July, 1960, 100: 7–15. (*Vanderbilt U. School of Med., Nashville, Tenn.)

Seventy-three infants acutely ill with clinical bronchiolitis were studied with special interest focused on cultivation of *H. influenzae* and demonstration of type-specific agglutinating antibodies in convalescent serum. Typable strains were identified in 35 (48 per cent), while in 13 (17.8 per cent), nontypable strains were isolated. Agglutinating antibody became detectable against homologous types in 31 of the 35. Of the 13 with nontypable strains, 11 developed type-specific agglutinating antibody. Of 23 who grew no *H. influenzae*, 17 developed type-specific agglutinating antibody. *Diplococcus pneumoniae* was identified on blood-agar plates from 21 patients. Cross-reactions between these two microorganisms are discussed. Nasopharyngeal washings from 46 infants failed to reveal any cytopathologic agents in monkey kidney or HeLa cell cultures. Hemadsorption tests on cultures from 24 patients were all negative. Paired serum samples from 73 patients failed to show rising C-F titers for influenza virus (a, b, c, and d) or adenovirus, CA, HA-1, or HA-2. One child had a rising titer to influenza virus, type a. It is probable that bronchiolitis, like croup, is associated with a variety of etiologic agents, "*H. influenzae* pneumonitis" being one form indistinguishable clinically from the others. In any case, the evidence presented in this report implicates *H. in-*

fluenzae as an active agent which may be associated with acute bronchiolitis in infants. It would seem logical, therefore, to direct antimicrobial therapy of patients toward its elimination. —M. J. Small

Interstitial Pneumonitis Induced by Experimental Infection with *Hemophilus Influenzae*. S. H. W. Sell* and J. L. Shapiro. *AMA J Dis Child*, July, 1960, 100: 16–22. (*Vanderbilt U. School of Med., Nashville, Tenn.)

Bronchiolitis with interstitial pneumonia was demonstrated in microscopic sections of suckling mice after intranasal inoculation with virulent *H. influenzae*, types a, b, and c. The bacilli were isolated originally from infants acutely ill with bronchiolitis and were recovered on lung cultures from the majority of the mice. Three different types of *H. influenzae* produced identical pathologic lesions. The addition of bacteria-free filtrate of nasopharyngeal mucus from the original patients did not appear to influence the pattern of the pneumonitis produced by the *H. influenzae* alone. The filtrate alone did not produce detectable pathologic lesions. The results indicate that *H. influenzae* types a, b, and c, isolated from infants with acute bronchiolitis, are capable of producing interstitial pneumonitis and bronchiolitis in newborn mice. The pathologic changes produced are similar to the lesions reported in the lungs of infants who died of the disease. This information supports the bacteriologic and serologic studies which have implicated *H. influenzae* in the pathogenesis of bronchiolitis.—M. J. Small

Massive Thoracic Extramedullary Hemopoiesis. R. B. Hanford*, G. F. Schneider, and J. D. MacCarthy. *New Engl J Med*, July 21, 1960, 263: 120–123. (*V.A. Hosp., Spokane, Wash.)

Two patients seen during the past 10 years serve to emphasize the fact that paravertebral masses of hemopoietic tissue in the thorax can develop in hereditary spherocytic anemia. In the 2 cases described, the significance of the anemia had been overlooked and the patients were referred for investigation of what was assumed to be primary neoplasms in the thorax. In both cases successful therapy depended upon identification of the primary process, followed by splenectomy. Both patients responded favorably to splenectomy, but the masses in the chest have not regressed. Evidence from a reported autopsy case indicates that these masses may be expected to remain as active hemopoietic tissue. Four autopsy cases mentioned in the literature are discussed.—M. J. Small

Idiopathic Pulmonary Hemosiderosis Presenting with Spontaneous Pneumothorax. K. H. Nickol*. *Tubercle*, June, 1960, 41: 216–218. (*Brompton Hosp., London, England)

Idiopathic pulmonary hemosiderosis is an uncommon condition which usually presents with recurrent hemoptyses, dyspnea, and progressive mottling in chest roentgenograms. A case presenting with a spontaneous pneumothorax is described. No previous record has been found of the association of the condition with spontaneous pneumothorax. The case contributes no fresh evidence concerning the cause of the condition.—M. J. Small

Hippocratic Fingers and Hypertrophic Osteoarthropathy: A Study of 350 Cases. C. Coury*. *Brit J Dis Chest*, July, 1960, 54: 202–209. (*Hotel Dieu, Paris, France)

Clubbing of the digits is a feature common to simple "hippocratic fingers" and to "hypertrophic osteoarthropathy." Although closely connected, the 2 conditions must be considered separately from several aspects. The former (H.F.) is a fairly frequent symptom, usually symptomatic of various chronic respiratory diseases, congenital heart defects with cyanosis, bacterial endocarditis, primary or secondary polycythemia; it may also develop occasionally with cirrhosis of the liver in children, chronic intestinal disease and diarrhea, gastric stenosis, et cetera. The latter (H.O.A.) is much less frequent. It includes other symptoms besides clubbing: enlargement of the extremities, articular pain and swelling and, above all, extensive periosteal thickening of the diaphysis of the bones. Although H.O.A. may be associated with any of the same thoracic and extrathoracic diseases as is simple H.F., malignant chest tumors account for more than 90 per cent of all typical cases. H.F. and H.O.A. belong to a common group of peripheral disorders. Together wtih pachydermoperiostitis they may be idiopathic, but all are highly significant of visceral, particularly intrathoracic, disease. A review is made of 350 cases of these so-called dysacromelias. The thoracic disease usually precedes the dysacromelia, and the progress of the 2 conditions is closely associated. The pathogenesis of dysacromelias and their mechanism are not yet established.—M. J. Small

Pulmonary "Coin" Lesion of Unusual Pathology. R. Paul*. *Radiology*, July, 1960, 75: 118–120. (*Pneumoconiosis Medical and Research Bureau, Kitwe, Northern Rhodesia)

A "coin" lesion was demonstrated in the chest roentgenogram of a European miner in South Africa. On pathologic section postsurgically, the tumor consisted of infarcted pulmonary tissue with thrombosed vessels and an intra-arterial schistosome.—W. J. Steininger

The Fatty Acid Composition of Human Thoracic Duct Lymph Lipids. R. Blomstrand* and O. Dahlbäck. *J Clin Invest*, July, 1960, 39: 1185–1191. (*U. of Lund, Sweden)

Analyses were made of the fatty acid composition of the lipid components of human thoracic duct lymph by gas liquid chromatography. The fatty acid distribution in the lymph triglycerides and the cholesterol esters was largely influenced by the dietary fat. The free fatty acids had quite another pattern than did the lymph triglycerides and were not influenced by the dietary fat. The phospholipid fatty acids were influenced very little by the dietary fat.—E. Dunner

Chronic Obstructive Pulmonary Disease in Young Adults. E. H. Kabon*, G. A. Koelsche, and W. S. Fowler. *Proc Mayo Clin*, June 8, 1960, 35: 307–316. (*Mayo Clinic, Rochester, Minn.)

The clinical records were studied of 102 males and 98 females 15 to 49 years of age who showed objective evidence of chronic obstructive pulmonary disease. Particular attention was paid to the clinical background of respiratory infection, allergic disease, and smoking habits. Evidence of asthma, infection, or both was found in about 80 per cent of all females and in the males up to 35 years of age. Of males in the age group 35 through 49 years, 40 per cent did not give a history of asthma or well-defined pulmonary infections but, with few exceptions, they were moderate-to-heavy cigarette smokers. The etiologic factors of chronic obstructive emphysema remain obscure but may well be multiple and may vary with the age of the patient (after authors' summary).—E. A. Riley

Middle Lobe Syndrome. E. Laustela*. *Ann Chir Gynaec Fenn*, Fasc. 2, 1960, 49: 160–168. (*Kirurgi, Helsinki, Finland)

In a series of 23 cases of middle lobe syndrome, the etiology was tuberculosis in 5 cases, foreign body in 2 cases, a broncholith in 1 case, and nontuberculous respiratory infections in the remaining 15 cases. The most frequent symptoms were productive cough, fever, hemoptysis, chest pain, dyspnea, and easy fatigability. Nine patients were treated surgically without postoperative complications. On follow-up examination, 7 were asymptomatic, but 2 complained of slight respiratory troubles without roentgenographic abnormalities. Of the conservatively treated 14 patients, 3 had frequent respiratory infections, but 11 were symptomless.—J. Haapanen

Mucormycosis. R. McBride*, J. Corson, and G. Dammin. *Amer J Med*, May, 1960, 28: 832–846. (*Harvard Med. School, Boston, Mass.)

Two cases are presented of mucormycosis, a usually fatal infection reported with increasing frequency. In 1 patient, a young woman with postabortal necrosis of the spleen, kidneys, and adrenals and severe uremia, mucormycosis was thought to contribute to the fatal outcome. The second case was in a young man with hepatic disease, possibly toxic in origin, and ischemic tubular necrosis. In both cases the mucormycosis was disseminated, involving the respiratory and gastrointestinal tracts. The clinical and pathologic features of 55 cases of mucormycosis reported in the English literature are summarized. In 21, diabetes was present, and in 13, leukemia or lymphoma. The role of these conditions, as well as that of cortisone and antimicrobials in the pathogenesis of mucormycosis, is discussed. The most frequently involved sites have been the brain and meninges, usually in diabetic patients. Pulmonary and disseminated forms have predominated in nondiabetic subjects. The diagnosis of mucormycosis should be suspected in the diabetic patient who presents a triad of orbital cellulitis, ophthalmoplegia, and meningoencephalitis. Some of these patients may have ulceration of the nasal mucosa, and here the diagnosis may be established by histologic examination and culture of biopsy material or scrapings. The diagnosis in the pulmonary and gastrointestinal forms of infection remains difficult. In the pulmonary form, a primary pneumonitis occurs. The spores are inhaled, penetrate the bronchial wall, and invade hilar tissues, parenchyma, and blood vessels, causing mucorthrombosis and infarction of the pulmonary parenchyma. Changes in the lung indistinguishable from the primary form may result from hematogenous or lymphatic spread of organisms from remotely infected tissue. In the gastrointestinal form, fungi in the lumen penetrate the bowel wall and invade mesenteric vessels, producing ischemic infarction and making generalized dissemination possible.
—T. H. Noehren

Clinical Investigation of Paragonimiasis. J. Eikas* and P. K. Kim. *Acta Tuberc Scand*, No. 2, 1960, 39: 140–147. (*National Medical Center, Seoul, Korea)

Paragonimiasis, a serious health problem in Korea, is caused by the trematode *Paragonimus westermani*, found in raw or improperly cooked fresh-water crabs and crayfish. It is a disease of varying severity, but tends to be particularly severe in young children. In a clinical investigation of 27 patients with proved pulmonary paragonimiasis, persistent cough with rust-colored scanty sputum was the most characteristic symptom. Neurologic signs and symptoms were frequent. Thin-walled cystic shadows are typical roentgeno-

graphic signs. The diagnosis of pulmonary paragonimiasis is proved by demonstration of ova in the sputum, gastric lavage, or feces. Treatment is unsatisfactory.—W. J. Steininger

Hemorrhagic Pericardial Fluid in Acute Benign Nonspecific Pericarditis: Report of a Case and Review of the Literature. C. Williams, Jr.*, J. Beckwith, and J. Wood, Jr. *Ann Intern Med*, April, 1960, 52: 914–923. (*U. of Virginia, Charlottesville)

Reports are available of 22 cases, including the authors', in which pericardial taps were done in acute benign nonspecific pericarditis. Of these 22 cases, one was a dry tap and a second was described as blood-tinged, but was thought to be due to a previous traumatic tap. Of the remaining 20, 14 were hemorrhagic, varying from a few red blood cells to very bloody, with a hematocrit of 10 per cent in 1 and 6 per cent in 2 others. Several authors have commented upon the hemorrhagic nature of the pericardial fluid, but only recently has some emphasis been placed on this point. Effusions during the first 10 to 14 days of illness are usually serous, but the fluid obtained later in the course of the disease is serosanguineous. The authors tend to agree with this, but have found 2 cases reported as serosanguineous before the 10th day: one on the 4th day and the other on the 5th day of illness; and a 3rd case described as serosanguineous on the 11th day. The few reports given, with still fewer details as to the day of illness when the tap was done, do not allow a more definite conclusion on this point. The realization that hemorrhage into the pericardium does occur in acute benign nonspecific pericarditis seems to have at least 2 very practical applications: (*1*) anticoagulants are contraindicated in this disease, which is often mistakenly diagnosed as myocardial infarction, and (*2*) bloody pericardial fluid does not necessarily mean more serious disease, such as tuberculous pericarditis or even effusion due to malignant neoplasm.—T. H. Noehren

Staphylococcal Pneumonitis in the Postoperative Patient. E. Morgan*, L. Lancaster, H. Pearsall, and G. Lawrence. *Dis Chest*, July, 1960, 38: 1–12. (*Mason Clinic, Seattle, Washington)

Because of its high mortality, staphylococcal pneumonitis in the immediate postoperative course commands special attention. On the basis of 13 such cases, seen from 1955 through 1958, an intensive course of therapy is described. Unlike the acute bacterial infections which can be identified readily, those due to staphylococci can be most difficult to recognize. Signs of severe respiratory insufficiency, low temperature, prolonged expiration despite gross tachypnea and hyperpnea, purulent bloody sputum, and leukocytosis lead to the suspicion of staphylococcal pneumonia; there is frequently lack of confirmatory roentgenographic changes in the lungs. A Gram stain of the sputum is sufficient basis to initiate vigorous therapy without delay. The intravenous use of antimicrobials plus all the other accepted respiratory aids, including tracheotomy, have proved their value.
—E. A. Rouff

Relapsing Polychondritis. C. M. Pearson*, H. M. Kline, and V. D. Newcomer. *New Engl J Med*, July 14, 1960, 263: 51–58. (*U. of California School of Med., Los Angeles)

Two cases of an entity called relapsing polychondritis are described in detail. Two other patients with focal chondritis and features of other disease are briefly presented. In essence, this is a multiple recurring disease of cartilage in which chondrolysis causes clinical arthritis, collapse or distortions of the external ears and the cartilaginous portion of the nose, dissolution of portions or all of the cartilages of the trachea, bronchi, larynx, and epiglottis, as well as occasionally those of the costal cartilages and the spinal articulations. Its etiology and pathogenesis are unknown, and its relation, if any, to 1 of the connective tissue (collagen) diseases or to the cartilaginous dystrophies is indistinct at present. Because of the exuberant granulation tissue that may appear in the respiratory tract, severe pulmonary symptoms (suffocation or infection) are common. Corticosteroids have been very effective in controlling the clinical symptoms and the progression of the disease. The differential diagnosis is discussed in some detail, and the 10 cases previously described in the literature are briefly summarized.—M. J. Small

Pulmonary Alveolar Proteinosis—A New Disease? H. P. Plenk*, S. A. Swift, W. L. Chambers, and W. E. Peltzer. *Radiology*, June, 1960, 74: 928–937. (*St. Mark's Hosp., Salt Lake City, Utah)

Pulmonary alveolar proteinosis is characterized by insidious onset, fatigue, weight loss, progressive dyspnea, cough, and slight fever. Roentgenographic findings vary from fine nodular to feathery consolidation localized to isolated lung segments or to widespread bilateral, often symmetrical, confluent densities involving an entire lung. The 4 cases here reported bring the total reported in the literature to 35, with a mortality rate of 34 per cent. Four of 9 patients had positive reactions to a complement-fixation test with an antigen from a fatal case of Pneumocystis pneumonia. It is speculated that the cause of the disease may be an organism, possibly antigenically related to *Pneumocystis carinii*, rather than inhalation of new volatile

agents. The possibility of some virus being the cause of both pulmonary alveolar proteinosis and Pneumocystis pneumonia has not been ruled out definitely. The possible role of antimicrobials in the etiology of the disease is discussed.
—W. J. Steininger

Pulmonary Alveolar Proteinosis and Nocardiosis. B. Burbank*, T. Morrione, and S. Cutler. *Amer J Med*, June, 1960, 28: 1002–1007. (*State U. College of Med. at New York, N.Y.)

A case is reported of pulmonary alveolar proteinosis complicated by suppurative pneumonia due to *Nocardia brasiliensis*. The nocardiosis was successfully treated with penicillin, sulfadiazine, and streptomycin. The lesions due to pulmonary alveolar proteinosis disappeared, as judged by roentgenographic study, during the course of 2 years. The pulmonary status of the patient demonstrates a restrictive disability, perhaps secondary to scarification and fibrosis following the resorption of the pulmonary alveolar protein complex.
—T. H. Noehren

Physiological and Clinical Aspects of Pulmonary Alveolar Proteinosis. W. Fraimow*, R. Cathcart, and R. Taylor. *Ann Intern Med*, June, 1960, 52: 1177–1194. (*Jefferson Med. College, Philadelphia, Pa.)

Two patients with pulmonary alveolar proteinosis are reported. Both alveolar-capillary block and significantly increased venous admixture were found to be responsible for the cyanosis in these patients. These findings, in conjunction with the available information in the literature, appear to demonstrate conclusively that this is the mode of production of the severe pulmonary insufficiency frequently found in this disease. At the present time there are no other apparent specific therapeutic measures that can be employed.
—T. H. Noehren

A Study of the Aetiology of Respiratory Disease in a General Hospital. W. W. Holland*, E. I. Tanner, M. Pereira, and C. E. D. Taylor. *Brit Med J*, June 26, 1960, No. 5190: 1917–1923. (*St. Thomas's Hosp. Med. School, London, England)

During the winter of 1958–1959, bacteriologic and virologic investigations were made on all patients (179 adults and 117 children) who were admitted with acute respiratory illness to the medical wards of St. Thomas's Hospital. Twenty-eight per cent of the illness in the children and 22 per cent of that in the adults was associated with an identifiable virus. Influenza A and B viruses were those most often found. The para-influenza groups of viruses were responsible for very few infections. *Streptococcus pneumoniae* was isolated from 36 per cent of the samples of sputum tested, *Hemophilus influenzae* from 22 per cent, and *Staphylococcus aureus* from 2 per cent. No relationship was found between the presence of particular bacteria in the nasopharynx or sputum and evidence of virus infection. No deaths occurred among the children, but among the adults there were 12 (6.7 per cent). Seven patients (2 children and 5 adults) developed staphylococcal pneumonia after admission, and in 6 of these patients the pneumonia was due to a strain of *S. aureus* acquired from the hospital environment. Of the patients studied, it was found that 24 per cent of the children and 33 per cent of the adults acquired a new strain of *S. aureus* during their stay in hospital. Most of these strains were resistant to more than one antimicrobial. The survey illustrates that respiratory disease accounts for more than ⅓ of all child admissions and at least 15 per cent of all adult admissions to a general hospital in the winter. The etiology of these infections could be determined in about ¼ of the patients admitted (after authors' summary).
—E. A. Riley

Familial Sarcoidosis. A Report of Two Cases. R. K. Merchant* and J. P. Utz. *AMA Arch Intern Med*, July, 1960, 106: 64–70. (*Infectious Disease Service, Dept. of Health, Ed., and Welfare, Washington, D.C.)

Sarcoidosis was observed in a 57-year-old Negro mother and her 33-year-old daughter. These and the 73 other cases of sarcoidosis reported in more than 1 member of 32 families suggest the possibility that a complex hereditary trait is operative in the pathogenesis of sarcoidosis.
—W. J. Steininger

The Resemblance between Sarcoidosis with Hypercalcaemia and Hyperparathyroidism. P. Ellman* and A. M. Parfitt. *Brit Med J*, July 9, 1960, No. 5192: 108–110. (*St. Stephan's Hosp., London, England)

The case is presented of a patient with sarcoidosis and hypercalcemia in whom decreased tubular reabsorption of phosphate was demonstrated, producing a biochemical resemblance to primary hyperparathyroidism. The differential diagnosis between the two conditions is discussed, and it is concluded that the tests of phosphate excretion may be misleading, but that the response to cortisone gives valuable information (Authors' summary).—E. A. Riley

Reticulosarcoma Complicating Sarcoidosis. R. M. Buckle*. *Tubercle*, June, 1960, 41: 213–215. (*St. Bartholomew's Hosp., London, England)

In this case report, the early features of this female patient's illness were entirely typical of sarcoidosis. As far as could be determined, they did not extend beyond the lungs and they resolved roentgenographically while under observation and without treatment. Microscopic examination at necropsy confirmed the diagnosis. At the time that the sarcoid granulomas were regressing, the patient developed a reticulosarcoma: this originated in multicentric sites, suggesting that the reticuloendothelial system was simultaneously involved over a wide field. At post-mortem examination there was no evidence of any sarcomatous infiltration of the lungs. The interesting speculative feature of this case is whether some unknown stimulus was responsible for both the initial sarcoid granulomatous reaction and the later sarcomatous metaplasia.—M. J. SMALL

Irreversible Renal Failure Following Short-Term Therapy in Sarcoidosis. H. S. BALLARD*. *AMA Arch Intern Med*, July, 1960, 106: 112–116. (*V.A. Hosp., 1st Ave. at 24th St. New York)

This report presents a case of sarcoidosis in which irreversible renal damage occurred after administration of 1,200,000 units of vitamin D (in the form of calciferol) over an 8-day period. Ectopic calcification, renal calculi, and nephrocalcinosis appeared as complications. Steroid therapy was effective in reducing the ectopic calcium deposited, but there was little improvement of renal function. An attitude of caution, perhaps of prohibition, in the use of calciferol in sarcoidosis is indicated. Small doses ingested over short intervals may result in serious and irreversible damage. Toxic reactions may be more frequently encountered during periods of enhanced exposure to sunlight. Interruption of vitamin D therapy at the earliest sign of intoxication may not avoid delayed renal damage. Proteinuria, pathologic urinary sediment, and nitrogen retention may persist indefinitely. —W. J. STEININGER

A Case of Pulmonary Tularemia Operated on for Suspicion of Bronchogenic Carcinoma (in German). C. CORONINI*, W. KOVAC, and G. SALZER. *Wien Klin Wschr*, July 22, 1960, 72: 531–536. (*Hygiene-Institut der Universität, Vienna, Austria)

A 58-year-old man, a heavy cigarette smoker, developed cough and subfebrile temperatures. Roentgenographic examination revealed a small, sharply delineated infiltration near the upper right hilum. Tomography showed this infiltrate to cause narrowing of the apical segmental bronchus and revealed the presence of a tracheobronchial lymph node. The diagnosis of bronchogenic carcinoma was made and a right upper lobectomy was performed. Histologic examination showed the presence of granulomatous tissue. Then the serum of the patient was examined for agglutination of *Bacterium tularense* and was found positive to 1:5,000. This, therefore, is a case of pulmonary tularemia, which presented clinically and roentgenographically as a typical bronchogenic carcinoma.—G. C. LEINER

TUBERCULOSIS, PULMONARY

A New Face of Tuberculosis. F. T. ROQUE*. *Amer J Med Sci*, July, 1960, 240: 17–20. (*U. S. Army Hosp., Huntsville, Ala.)

As a result of the use of high doses of isoniazid (10 to 16 mg. per kg. of body weight) in combination with daily PAS or streptomycin, or both, complete clearing of tuberculous pulmonary infiltrations may occur in about 30 days. This is a new face of tuberculosis. Physicians are cautioned not to rule out the diagnosis of tuberculosis just because disappearance of the pulmonary infiltrations occurs as fast as with bacterial pneumonia. Three such cases of pulmonary tuberculosis and one pleural effusion, all of which cleared rapidly, are presented.—W. J. STEININGER

Bacteriological Aspects of the So-Called Tuberculomas of the Lungs. G. SANTOPEDRE* and P. F. DELLE SEDIE. *Acta Tuberc Scand*, No. 2, 1960, 39: 97–114. (*U. of Pisa, Italy)

Bacteriologic examinations were carried out on 22 resected tuberculomas from 13 subjects. Tubercle bacilli were demonstrated on direct examination in 11 (50 per cent) of the 22 tuberculomas and by culture in 13 (59 per cent), but there was little correlation between the 2 methods. Growth, when it occurred, was often sparse, leading to the conclusion that in these tuberculomas a large proportion of visible microorganisms are not alive or cannot reproduce themselves either *in vitro* or *in vivo*. The fate of tubercle bacilli in tuberculomas is related to the age and pathologic nature of the lesions. It is possible to find in the tuberculomas bacilli with various degrees of viability and pathogenicity.—W. J. STEININGER

Localized Pleural Tuberculosis (in Japanese). R. SATO*, C. TODA, M. HUJIHARA, and C. KUEN. *Jap J Thor Surg*, July, 1960, 13: 570–575. (*Kobe Med. College, Japan)

Five cases are described of so-called encapsulated pleurisy marked by roentgenographic findings of round or semilunar homogenous shadows adherent to or with a base to the thoracic wall. Thin-walled caseous lesions localized between the visceral and parietal pleuras were found at sur-

gery; they were apparently different from tuberculous intercostal lymphadenitis or localized pyothorax, both anatomically and pathologically, and the writers propose to call them "localized pleural tuberculosis." All of the patients were in the third or fourth decades of age, and 3 of the 5 patients had histories of either tuberculous lesions of other organs, or pleurisy.—I. TATENO

Liver Function Studies in Pulmonary Tuberculosis (in German). E. KLAPÖTKE*. *Tuberkulosearzt*, May, 1960, 14: 278–285. (*Heilstätte Schwarzenbach, Clausthal-Zellerfeld, Germany)

Liver function was studied in 2 groups of tuberculous patients (50 and 70 patients) before and after 8 weeks of treatment with thiosemicarbazone and isoniazid. The second group received in addition Litrison®, a mixture of methionine, choline, vitamin B complex, and vitamin E. The degree of dysproteinemia reflected the severity of the hepatic involvement caused by tuberculous toxicity. Chemotherapeutic drugs, especially thiosemicarbazone, and surgical measures caused impairment of liver function. Small doses of Litrison during chemotherapy and before and after surgery prevented hepatic lesions to a large extent. Deterioration of liver function during treatment should be counteracted by administration of large doses of Litrison.—J. HAAPANEN

Pathologic-Anatomic Studies on Resected Lungs of Younger Patients with Pulmonary Tuberculosis (in Japanese). Y. NAGAKURA*. *Clin Resp Org (Tokyo)*, July, 1960, 15: 462–474. (*National Kiyose Sanatorium, Japan)

Pathologic-anatomic studies were made on 88 lung specimens obtained at surgery from patients aged between 8 to 17 years. Female patients predominated in the age group of 12–17 years; the anatomic findings in these patients usually consisted of caseous bronchitis, and the tuberculomas usually contained caseous foci in the center. Cavities or main foci of the lesion were present in the upper lobe in 64 patients, in the lower lobe in 9, and in all 3 lobes in 15. Cavitation of the primary focus of infection was seen in 3 cases. Evidence of severe lesions in the bronchus was observed in a patient who had clinical findings of epituberculosis in the course of the illness, while another patient who had a middle lobe syndrome had a middle lobe bronchus occluded by caseous lesions. Of the 87 cavities studied, chemotherapy was considered to have brought about the following changes: complete inspissation, 1; nearly complete inspissation, 16; occlusion of the cavity space with caseous material, 11; and shrinkage of the cavity, 13. The cavitary walls were thin in 7 cases, and a tendency to open healing of the cavity was seen in 13 cases, which included all of the former 7 cases. Open healing was seen in 5.1 per cent, and remarkably improved cavities were seen in 22 per cent. These findings indicate that the lesions in younger patients had a tendency to clear up more effectively than did the lesions in adults.—I. TATENO

Continued Observations on the Role of Steroid Therapy in Tuberculosis. A. MILLER*. *Dis Chest*, July, 1960, 38: 13–19. (*V. A. Hosp., Buffalo, N. Y.)

Over the past 4 years, 48 patients have been carried on a program of adequate antituberculosis chemotherapy plus steroids: 38 had far-advanced disease, 1 with meningitis; 6 had moderately advanced disease; and 4 had miliary tuberculosis associated with meningitis in 2 instances. Prednisone has been used in the majority of the cases: an initial dose of 60 mg. is reduced to 30 mg. over a 1-week period, continued at this level for 3 or 4 weeks, and then decreased so that the total steroid administration extends over 2 or 2½ months. Corticotropin gel in a dose of 20 units daily is started the final 3 days of the prednisone administration. Results have shown a prompt reversal of toxicity with loss of fever, gain of weight, and general sense of well-being. Roentgenographic improvement appears to be accelerated, and no adverse roentgenographic effects have been seen. Seven patients have undergone resection and 2 had thoracoplasties without difficulty. Drug susceptibility has been retained. The resected specimens showed no apparent differences from those from nonsteroid-treated cases.—E. A. ROUFF

TUBERCULOSIS, NONPULMONARY

"Chronic" Carcinoma of the Larynx (in German). O. NOVOTNY*. *Wien Klin Wschr*, June 3, 1960, 72: 403–404. (*II. Hals, Nasen-, Ohrenklinik, Universitat, Vienna, Austria)

A squamous cell carcinoma of the larynx was diagnosed in a 24-year-old woman. It was treated repeatedly with radiation (X-ray and radium) and observed for 28 years. This, therefore, is an unusual case of a minimally active, chronic carcinoma.—G. C. LEINER

The Problem of Optic Atrophy Complicating Tuberculous Meningitis. R. DES PREZ* and C. JORDAHL. *Ann Intern Med*, May, 1960, 52: 1118–1123. (*Cornell U. Med. College, New York, N.Y.)

The successful drug treatment of tuberculous meningitis has produced the new problem of chronic neurologic sequelae in some survivors. One of these is optic atrophy with blindness. Two cases

of this complication are presented. In the first case, persisting blindness was possibly related to delay in initiation of corticosteroid therapy; in the second, the reversal of blindness appeared to be related to the immediate use of these hormones. The pathology of tuberculous meningitis would indicate that blindness may be due to chiasmal compression from the hyperplastic inflammatory tissue at the base of the brain. It is suggested that blindness complicating tuberculous meningitis be considered an indication for immediate institution of adjuvant hormone therapy, and that craniotomy with chiasmal decompression be considered in cases not responding to this treatment (Authors' summary).—T. H. NOEHREN

Tuberculous Primary Infection in Pregnancy and Its Relation to Congenital Tuberculosis. R. GRENVILLE-MATHERS*, W. C. HARRIS, and H. J. TRENCHARD. *Tubercle*, June, 1960, 41: 181–185. (*Edgware General Hosp., Middlesex, England)

The multiple-puncture tuberculin test was given to 3,855 pregnant women; 1,580 (41 per cent) had negative reactions and 1,207 of these were retested later in pregnancy; 33 were found to be positive at the second test, 2 as a result of BCG vaccination. None of the children of these 33 women developed congenital tuberculosis. No tubercle bacilli were cultured from the breast milk or lochia, but bacilli were cultured from 2 placentas. Congenital tuberculosis can occur with comparatively slight maternal disease and, presumably, is associated with genital or placental infection, but such infection does not invariably cause congenital tuberculosis. Infection of the placenta by tubercle bacilli is a possible but not necessarily frequent consequence of primary infection in pregnancy. To the naked eye, an infected placenta may appear quite normal, and histologic changes may be absent.
—M. J. SMALL

THORACIC SURGERY

Bronchiectasis. O. PERÄSALO*, T. M. SCHEININ, and P. PANTZAR. *Ann Chir Gynaec Fenn*, Fasc. 2, 1960, 49: 139–159. (*Kirurgi, Helsinki, Finland)

II. The Surgical Treatment of Bilateral Disease: pp. 139–149:

A series is reported of 117 patients with bilateral bronchiectasis, treated from 1947 through 1958. Fifty-eight (49.6 per cent) of the patients were operated on. Unilateral resection was performed in 47 cases, and bilateral in 11 cases. Symmetric bronchiectatic changes were present in 74 per cent of all cases. Unilateral resection was performed on the left in 83 per cent and on the right in 17 per cent. The complication rate has been decreasing constantly. In symmetric disease the mortality was 9 per cent after unilateral and 18 per cent after bilateral resection. Pulmonary edema was the most common of the serious complications after bilateral resections.

III. Bronchiectasis due to Foreign Bodies: pp. 150–159:

Eight cases of bronchiectasis due to foreign bodies are reported. They represented 3.7 per cent of a series of 215 cases of bronchiectasis. Seven cases were due to aspiration of foreign bodies, usually at the age of 1 to 2 years. The typical early symptoms were followed by an average period of 20 years with varying inflammatory symptoms. Frequent hemoptysis was characteristic. In 6 cases the foreign body was situated in the basal segments of the right lower lobe, and in 2 it was in the left lower lobe. In 3 cases the foreign body was a spike of rye, and in 1 case each it was the tip of a spruce twig, timothy grass, a chip of wood, a ball of paper, and a penetrating metal fragment. The early and late results of the operation were excellent and definitely superior to those obtained in other forms of bronchiectasis. In 6 patients bronchography showed no recurrence of bronchiectatic changes after an average follow-up period of 5.2 years. It is emphasized that early diagnosis and treatment are essential in these cases, as they might prevent the development of severe and disabling pulmonary changes.—J. HAAPANEN

General Anesthesia for Bronchoscopy with Special Reference to the Use of Eunal® (in Japanese). K. INADA* and N. SATO. *Jap J Thor Surg*, July, 1960, 13: 565–569. (*Okayama U. School of Med., Japan)

A general anesthetic technique for bronchoscopy is described. Anesthesia was induced with a new nonbarbiturate intravenous anesthetic agent, Eunal®, followed by succinylcholine chloride. After the lungs were ventilated with pure oxygen for a few minutes, a specially devised bronchoscope was introduced and a mixture of nitrous oxide and oxygen was administered through the side arm of the bronchoscope. With this technique, examinations could be performed with complete muscular relaxation without unnecessary haste while breathing was controlled with a manual "pressure bag." The time required for operation on one patient and care of the postoperative patients did not exceed that required when only local anesthesia was used. The present method has been used satisfactorily in 46 patients.—I. TATENO

Clinical Experience With Chloroform Anesthesia. M. POE* and J. MAYFIELD. *Anesthesiology*,

No. 5, 1960, 21: 508-511. (*City of Memphis Hospitals, Tenn.)

This study was done in an effort to reassess the application and usefulness of chloroform combined with other anesthetics and adjuvants as a nonflammable technique. Seventy patients were carefully selected and used in the study. The method of handling these patients during the various operative procedures is discussed in detail. Chloroform used in combination with other drugs by nonflammable techniques was found to be useful particularly in short procedures requiring minimal muscular relaxation. The anesthesia requires care in administration and the observance of certain precautions: use of atropine or scopolamine premedication; induction of anesthesia with intravenous thiobarbiturate; avoidance of sudden increase in concentrations of the vapor; use of combined techniques and limit of drug concentration to the range of 0.5-1 per cent; ensurance of carbon dioxide removal; and avoidance of use in patients with liver damage.—E. Shabart

Bronchiolar Carcinoma (Pulmonary Adenomatosis). R. Southworth* and A. Becker. *Ann Intern Med*, June, 1960, 52: 1328-1337. (*Receiving Hosp., Detroit, Michigan)

A case is reported of bronchiolar carcinoma proved by biopsy at operation. The lesion had been present for 7 years as confirmed by serial roentgenograms. The long duration of the localized lesion suppo.ts the unicentric origin of this tumor. An isolated nodule is the early and asymptomatic phase of the disease. A review of all patients with bronchiolar cell carcinoma up to 1957 revealed only 4 proved cases surviving longer than 5 years without surgery. This is the fifth patient surviving longer than 5 years without surgery as proved by chest films, and the first case to survive 6½ years following histologic proof of the diagnosis.—T. H. Noehren

Esophagogenic Mediastinal Cysts (in German). E. Merikallio*. *Ann Chir Gynaec Fenn*, Fasc. 2, 1960, 49: 232-239. (*Kirurgi, Helsinki, Finland)

Two patients with esophagogenic mediastinal cysts were operated on. One was a 46-year-old female, and the other an asymptomatic 20-year-old soldier in whom the cyst had been diagnosed 10 years earlier. A follow-up examination showed both to be in good condition.—J. Haapanen

Cysts of the Lung in Infants and Children. S. Brunner*, P. T. Poulsen, and J. Vesterdal. *Acta Paediat (Upps)*, January, 1960, 49: 39-48. (*University Hosp., Copenhagen, Denmark)

A series is reported of 20 infants and children with cysts of the lung. Thirteen had histories of febrile pulmonary disease and 1 had had recurrent pneumothorax, whereas 6 had not presented any symptoms or signs until 1 month before admission. The roentgenographic findings are reviewed. It is concluded that it is impossible to decide roentgenographically, clinically, or by histologic investigation whether the cysts are acquired or congenital. Pneumonectomy was performed in 1 case, lobectomy in 9 cases, segmental resection in 6, and enucleation in 3. Surgery did not seem to be indicated in only 1 patient. Recurrent infections, progressive enlargement of solitary cysts, or rupture of large or small subpleural cysts afforded obvious indications for surgery. On the other hand, expectant attitudes seem justifiable in cases of small cysts detected by chance which show no increase in size and are not associated with infection. All of the surgically-treated patients included in the follow-up series were symptom-free except for 1 patient who was apt to become dyspneic on major exertion (after authors' summary). —H. J. Simon

Esophagotracheal Fistula after Blunt Thorax Trauma (in German). P. Brücke*. *Wien Klin Wschr*, July 1, 1960, 72: 482-483. (*Landeskrankenhaus Wagna, Steiermark, Austria)

Development of an esophagotracheal fistula is extremely rare. In a car accident, a 38-year-old man was thrown against the steering wheel. He developed severe chest pain, mediastinal emphysema, and dyspnea, and he expectorated food even many hours after eating. Esophagoscopy and bronchoscopy revealed the presence of an esophagotracheal fistula above the bifurcation. A jejunostomy was done for nutritional purposes. Thirty-two days after the accident, surgical repair of the fistula was done and resulted in complete recovery of the patient.—G. C. Leiner

Fluorescence Microscope Method for Detection of Pulmonary Malignancies. F. D. Bertalanffy*. *Canad Med Ass J*, July 30, 1960, 83: 211-213. (*U. of Manitoba, Winnipeg, Canada)

A method using fluorescence microscopy is described for the diagnosis of pulmonary malignancies by exfoliative cytology. This simple and rapid method introduces new diagnostic criteria of malignancy and facilitates recognition of malignant cells in sputum, bronchial secretions, and pleural fluids (after author's summary).—E. A. Riley

Hiatus Hernia. N. R. Barrett*. *Brit Med J*, July 23, 1960, No. 5194: 247-252. (*St. Thomas Hosp., London, England)

A comprehensive review is presented of the diagnosis, pathology, and treatment of hiatal hernia. —E. A. Riley

The Treatment of Pneumoconioses Complicated by Other Diseases of the Lungs (in French). Société de Chirurgie Thoracique de Langue Française*. *Poumon Coeur*, March, 1960, 16: Supplement. (*9 Avenue de la Bourdounais, Paris 7, France)

The entire edition is devoted to the treatment of pneumoconioses complicated by other diseases of the lungs: (*1*) In the surgical treatment of tuberculosis in the presence of silicosis or other pneumoconioses, the technique and the indications are generally the same as in uncomplicated tuberculosis. A special problem is presented by the fact that the pulmonary function of the lungs is more or less impaired by the pneumoconiosis. (*2*) Infection with unclassified tubercle bacilli in the presence of pneumoconioses is discussed, as well as (*3*) intracavitary aspergillosis in the presence of pneumoconioses. In general terms, the treatment is fundamentally the same as in uncomplicated cases. The prognosis is less favorable because of the loss of functioning lung tissue through the pneumoconiotic process and the progressive nature of this underlying condition.—E. Lyon

The Present Place of Monaldi's Drainage in the Treatment of Pulmonary Tuberculosis (in German). H. W. M. Rauch*. *Tuberkulosearzt*, May, 1960, 14: 290–303. (*Westerwaldklinik, Kr. Neuwied/Rh., Germany)

From 1938 to 1958, 169 patients with tuberculous pulmonary cavities were treated with Monaldi's drainage. The method was used alone in 30 cases, combined with thoracoplasty in 103 cases, and after failure of collapse procedures in 36 cases. It was concluded that this type of treatment is still of use in cases in which collapse therapy fails to produce cavity closure or when resection is not indicated or cannot be performed.—J. Haapanen

Surgical Treatment for Pulmonary Tuberculosis in Childhood. H. C. Nohl* and S. J. Steel. *Brit J Dis Chest*, July, 1960, 54: 255–264. (*London Chest Hosp., England)

Seventy-one children who were treated for pulmonary tuberculosis at High Wood Hospital between 1949 and 1958 and who had surgical treatment at the London Chest Hospital were selected for study. Eighteen were suffering from primary tuberculosis or its complications, and 53 had chronic pulmonary tuberculosis. In patients selected for surgery, resection is considered to be the method of choice, together with adenectomy in the case of enlarged glands causing symptoms. Extrapleural pneumothorax has been abandoned and thoracoplasty is contraindicated in children. The relative freedom from postoperative complications and the good roentgenographic results obtained by comparison with similar surgical treatment in adults are emphasized. There were no deaths. Only 1 child with primary tuberculosis failed to do well and developed renal and cervical glandular tuberculosis 3 years after operation. Among the children with chronic pulmonary tuberculosis treated surgically, only 2 had relapses, both following extrapleural pneumothoraces. The importance of adequate chemotherapy is stressed. It is concluded that surgical treatment should be confined to a small number of patients with persistent symptoms due to the various complications of primary tuberculosis, while in children with chronic pulmonary tuberculosis the indications for surgery are the same as in adults.—M. J. Small

250 Cases of Resection for Pulmonary Tuberculosis. H. J. Beard*. *Brit J Dis Chest*, July, 1960, 54: 265–271. (*Kelling Hosp., Holt, Norfolk, England)

A review is made of 250 cases of resection for pulmonary tuberculosis performed between March, 1950 and August, 1957. One of the surprises was the finding of how well most of the grave-risk pneumonectomy patients have done. Another has been that the segmental resection patients did well despite the fact that the drug treatment of the early cases was, by today's standards, very inadequate. The lobectomy figures are, however, disappointing in the matter of relapses. The number of relapses might have been reduced if more thoracoplasties had been done after the upper lobectomies. The over-all operative mortality was 6.4 per cent. Sixteen patients had a relapse of their disease at an average time of just over 2 years after operation. Bronchial fistula and empyema were the most serious operative complications. Although decreasing in use, resection still has a place in the treatment of pulmonary tuberculosis. It tends now to be reserved for those patients in whom, after chemotherapy, cavities or nodular areas of disease remain; but age and social and economic factors seem increasingly to influence the decision. There is, too, the occasional pneumonectomy still being performed on patients whose disease originated in the pre-streptomycin era.
—M. J. Small

Thymic Tumors (in German). T. K. I. Larmi*. *Ann Chir Gynaec Fenn*, Suppl. 93, 1960, 49: 1–47. (*Kirurgi, Helsinki, Finland)

Nineteen cases of thymic tumors represented 11 per cent of the surgically treated mediastinal tumors in this hospital; 12 were in females and 7 in males. One case was diagnosed at autopsy, and the remaining 18 histologically during operation. Two patients were symptomless, and 17 had either general symptoms or symptoms of compression. My-

asthenia gravis was seen in 4 cases. Routine roentgenography was normal in 3 cases, but revealed a tumor shadow adjacent to the superior anterior mediastinum. It was not possible to determine definitely by roentgenography the origin of the tumors. The tumors weighed from 18 to 250 gm. In 5 cases they were cystic, and in 3 cases there were also large thymogenic cysts. Invasive growth was seen in 8 patients. No distant metastases were encountered. In 1 case there was a thymolipoma. The diagnosis of hyperplasia of the thymus was made histologically in 4 cases, a lymphomatous thymoma was present in 9 cases, and an epithelial thymoma in 6 cases. In 12 cases the tumor could be removed surgically, but in 6 cases it was inoperable. Postoperative roentgen therapy was given in these 6 cases as well as in another 9 cases in which malignancy was suspected. On the follow-up examination, 9 patients were working, 8 had died, and 1 had recurrent myasthenia. The 1 tumor diagnosed at autopsy was found in a case of sudden death following thyroidectomy. Thymoma and hypoplasia of the adrenal gland were diagnosed at autopsy.—J. HAAPANEN

ROENTGENOGRAPHY

Anemia as a Presenting Symptom of Esophageal Hiatal Hernia of the Diaphragm. S. L. FELDER*, P. M. MASLEY, and W. I. WOLFF. *AMA Arch Intern Med*, June, 1960, 105: 873–883. (*St. Vincent's Hosp., N. Y., N.Y.)

Esophageal hiatal hernia is a common and important pathologic lesion and is a frequent etiologic entity for chronic blood-loss anemia, particularly in the aged, in whom it occurs with increasing frequency. Among a total of 267 cases of hiatal hernia diagnosed in a hospital population during the years 1950–1957, some degree of blood loss attributed to hiatal hernia alone was present in 61 (23 per cent). Of the total patient population with hiatal hernia, 10 per cent presented with hematemesis, 7 per cent with melena of a history thereof, and 6 per cent with an established blood-loss anemia but no or trace amounts of blood in the stool. Sixty-two per cent of the patients who had some degree of blood loss were more than 60 years of age. Emphasis is placed upon awareness of the lesion and its rising importance in gastrointestinal blood loss and the need for thorough roentgenographic examination to detect its presence.—W. J. STEININGER

Potassium Iodide Screens and High Capacity Roentgen Tubes in Angiocardiography. E. CARLSSON*. *Acta Radiol*, No. 6, 1960, 53: 481–485. (*Washington U. School of Med., St. Louis, Mo.)

A method is described in which exposure time is reduced without increasing the energy level of radiation required during angiographic examination. Thallium-activated potassium-iodide-intensifying screens in combination with high-capacity roentgen tubes permit optimal energy levels for these examinations. This method permits reduction in the concentration of iodinated contrast media. It decreases reaction risks and the dose of radiation. The drawbacks are the mottled films, such as are obtained with experimental screens, and the geometric blurring caused by large focal spots of high-capacity roentgen tubes.—E. SHABART

Application of Selective Pulmonary Angiography to the Study of Pulmonary Tuberculosis (in Japanese). K. KOBAYASHI*, S. TOMURA, J. YAZAKI, R. INOUE, and R. YOSHIMOTO. *Jap J Thor Surg*, July, 1960, 13: 576–580. (*Hinoso National Sanatorium, Gifu Prefecture, Japan)

The writers performed selective angiography on patients with pulmonary tuberculosis and obtained the following conclusions: There were several abnormalities in the pulmonary angiograms, namely, a diminution of vascularity, displacement, narrowing, occlusion and widening of the angle of bifurcation of the pulmonary arteries, and delayed circulation time in the lungs. The recognition of these findings was useful in the diagnosis of pulmonary tuberculosis. A capillary angiogram was considered to be indicative of the state of pulmonary function and was believed to show whether or not the functional capacity of the particular segment was reversible. The surgical method to be employed could also be determined by the findings of pulmonary angiography.—I. TATENO

Pulmonary Embolization Following Urethrography with an Oily Medium. A. H. ULM* and E. C. WAGSHUL. *New Engl J Med*, July 21, 1960, 263: 137–139. (*V. A. Hosp., N. Y., N. Y.)

Two patients are described who experienced pulmonary embolization as a complication of urethrography performed with Lipiodol®. In both, acute pneumonitis ensued. Pulmonary embolization, with resultant pneumonitis, is not a common complication of urethrography. The mishaps described should alert physicians to the risk that accompanies the use of oily media. The course of the pulmonary complication and the roentgenographic findings are not necessarily parallel. The persistence of roentgenographic changes long after subsidence of pulmonary symptoms suggests that a large part of the initial roentgenographic changes in the lung may be attributed to the radiopacity of the provoking material.—M. J. SMALL

Roentgenologic Studies of Respiration in Infants. J. F. Bosma, J. Fawcitt, J. Lind, Y. Takagi, and C. Wegelius. *Acta Paediat (Upps)*, March, 1960, 49: Supp. 123.

Part I: Disability of oral function in an infant associated with displacement of the tongue: This is a report of an infant who had a feeding disability and impairment of other oral functions similar to those impairments encountered in infants with hypoplasia of the tongue and/or palate. However, these structures were normal in the patient under consideration. It was inferred that the disability in this patient was due to inhibition of these oral performances incident to abnormalities in the cervical postural mechanism. It is proposed that normal suckling can only be performed against a background of regional inhibition of the musculature accomplishing anteroflexion of the head and neck. The particular clinical problems of this infant were relieved by a novel technique of prone position feeding.

Part II: Glossopharyngeal respiration as a part of focal seizures of the pharyngeal area in an infant: The rare and distinctive motor performance of glossopharyngeal respiration (GPR) is reported as a part of a seizure manifestation in a 1-month-old infant. This GPR is essentially identical with that learned spontaneously or by intention in some patients with poliomyelitis who have severe respiratory paralysis. It has also been observed as an isolated performance in the respiratory transition at the moment of birth. It is inferred that this is a mechanism or performance which the subject possesses potentially, but does not otherwise employ.

Part III: Roentgenographic observations of motions of the upper airway associated with establishment of respiration in the newborn infant: The whole of the respiratory organization is involved in the initial aeration of the lung and establishment of the respiration. The pharynx region may perform distinctively at this time in an engulfing action (glossopharyngeal respiration), the motions of which are highly similar to those of ingestion of a bolus into the esophagus. The more familiar respiratory modulations of the upper airway are also in action at this time, performing in relation to a comparatively stable hyoid base. These respiration-patterned motions may result in closure of the whole of the pharynx or the larynx. The mechanisms of elicitation of these varied actions of the upper airway, their immediate motor mechanisms, and their effect upon pulmonary ventilation require further investigation.—H. J. Simon

CHEMOTHERAPY

Tuberculosis Laboratory Methods. VA—Armed Forces Cooperative Study on the Chemotherapy of Tuberculosis*. July, 1960. (*V. A., Dept. of Med. & Surgery, Washington 25, D. C.)

These laboratory methods were compiled to serve as a guide for study unit laboratories in the Cooperative Study. The techniques and methods outlined were devised by many workers throughout the country; most of them are published in medical journals. This publication is not intended to replace standard textbooks or other laboratory and medical publications, but is provided to promote uniformity of method within VA—Armed Forces Study Unit Laboratories.

Acute Disseminated Coccidioidomycosis: Two Cases Treated with Amphotericin B. D. M. Perry* and W. M. M. Kirby. *AMA Arch Intern Med*, June, 1960, 105: 929-934. (*U. of Washington School of Med., Seattle)

Two patients with acute severe disseminated coccidioidomycosis made a dramatic initial response to therapy with amphotericin B. In both cases the drug was administered intravenously in large doses every second or third day for 4 to 5 months despite signs of toxicity (chills, fever, muscle aching, and rise in blood urea nitrogen). Corticosteroids were important in ameliorating side effects, thereby permitting prolonged intensive therapy. Both patients had relapses after apparent recovery; one responded poorly to re-treatment and died. These relapses, following excellent initial response, would seem to emphasize the need for more prolonged therapy.—W. J. Steininger

A Controlled Blind Study of Pneumococcal Pneumonia Treated with Tetracycline and Tetracycline Plus 6-Methyl Prednisolone. J. C. Kirby, Jr.*, G. Polis, and M. J. Romansky. *Amer J Med Sci*, July, 1960, 240: 30-35. (*D. C. General Hosp., Washington, D. C.)

A controlled blind study was carried out on 42 patients with pneumococcal pneumonia. The 21 patients treated with 6-methyl prednisolone in decreasing amounts over a 5-day period together with 2 gm. of tetracycline per day showed more rapid defervescence than did the control group treated with the same amounts of tetracycline alone. No "recurrence" or "rebound" of fever was noted upon steroid discontinuance, nor was there an instance of hypothermia with the initial dose of steroid therapy. Roentgenographic findings were inconclusive. Complications attributable to the 6-methyl prednisolone did not occur. There is no indication for adjunctive steroid therapy in uncomplicated pneumococcal pneumonia. Controlled studies may show the adrenal steroids to be of value in the highly febrile toxic patient.
—W. J. Steininger

Amino Acids in the Urine of Patients with Pulmonary Tuberculosis and the Effect of Isoniazid and Vitamin B_6 on Their Excretion (in Japanese). H. Kakinoki*. *Kekkaku*, July, 1960, 35: 499–502. (*Hokkaido U., Sapporo, Japan)

The writer studied the amino acid metabolism of patients with pulmonary tuberculosis by paper chromatography analysis. The concentration and number of amino acids excreted in the urine were usually higher in patients than in controls when the amino acid complex was injected intravenously, indicating lowered amino acid utilization by the patients. The number of ninhydrin-positive amino acids as demonstrated on paper chromatography increased on administration of isoniazid to the patients. They did not increase when vitamin B_6 was injected. The amino acids which increased on isoniazid medication were important ones such as lysine, methionine, phenylalanine, threonine, beta-aminoisobutyric acid, et cetera. The tendency of excreting more amino acids in the urine when they were injected intravenously was augmented by isoniazid administration and suppressed by vitamin B_6 administration; isoniazid and vitamin B_6 appeared to be competitive in human amino acid metabolism as well.—I. Tateno

Treatment of Pulmonary Tuberculosis in Diabetic Patients. O. T. Cheung*. *Canad Med Ass J*, July 16, 1960, 83: 118–120. (*Toronto Hosp. for Tuberculosis, Weston, Ontario, Canada)

The results are presented of treatment of 50 patients with pulmonary tuberculosis and diabetes mellitus. On admission, 54 per cent of the cases had far advanced tuberculous disease, 32 per cent had moderately advanced, and 14 per cent had minimal disease. All received combinations of antituberculous drugs; isoniazid was included in the drug regimen in all but 5. At the time of discharge of all cases from hospital, cavity closure was present in 82 per cent and sputum conversion in 93 per cent. In 88 per cent of the cases the results were considered satisfactory; that is, there was no cavity, the sputum was negative for tubercle bacilli, and the disease was stable roentgenographically. The results compare favorably with those of nondiabetic tuberculous patients (after author's summary).—E. A. Riley

A Retrospective Study of Cases of Pulmonary Tuberculosis Developing Drug Resistance. H. E. Thomas*. *Tubercle*, February, 1960, 41: 37–46. (*Birmingham Chest Clinic, England)

Using a definition based on minimal inhibitory concentrations, tubercle bacilli which had shown resistance to isoniazid, PAS, or streptomycin either singly or in combination were isolated from 182 patients with pulmonary tuberculosis treated with chemotherapy during 1956–1957. Sixty-one patients who had been reported since June 30, 1952, and who were prescribed combined chemotherapy with 2 or more drugs, were matched with the nearest bacteriologically proved patients of the same age and sex on the tuberculosis register in whom bacterial resistance had not been detected. These 122 cases were submitted to detailed retrospective study. The following findings were more common in the diagnostic roentgenograms of those patients subsequently showing bacterial drug resistance: an area of disease greater than the equivalent of 2 zones in the roentgenogram; and cavitation, especially if multiple, or if the diameter of the largest cavity was greater than 4 cm. across. Detailed analysis of the chemotherapeutic regimens showed a smaller proportion of cases in the drug-resistant group among the patients who had chemotherapy completely in hospital for the first 6 months of treatment. It also showed that there had been an increased use of daily PAS and isoniazid by outpatients in the isoniazid-resistant group, and of daily PAS and thrice-weekly streptomycin in the streptomycin-resistant cases. The implication of these findings is that the PAS was taken ineffectively and that the imperfect combined chemotherapy was responsible in some measure for the emergence of bacterial drug resistance. It is concluded that all patients having any of the above roentgenographic factors should have chemotherapy completely in hospital for at least 6 months if the emergence of drug resistance is to be avoided.—M. J. Small

Some Aspects of Drug Resistance in Tuberculosis and Its Treatment. S. Gillis*. *Med J Aust*, June 25, 1960, I: 1014–1019. (*Morris Hosp., Adelaide, Australia)

Patients with tuberculosis resistant to streptomycin, PAS, and isoniazid form a significant fraction of the admissions to chest hospitals in England and Australia. The extent of this problem and its treatment with new chemotherapeutic agents are discussed with particular reference to the role of isoniazid resistance from the individual and public health points of view.—H. J. Simon

Fulminating Purpura during Anti-tuberculosis Drug Treatment. P. A. Zorab*. *Tubercle*, June, 1960, 41: 219–220. (*Hampstead and St. Marylebone Chest Clinics, England)

The interest of the present case lies in the appearance of a fulminating purpura within a few hours of starting antituberculous drugs (streptomycin, PAS, and isoniazid), with death occurring within a few days. The chest roentgenogram taken shortly after admission showed evidence of gross bilateral cavitating tuberculosis and the sputum

contained numerous tubercle bacilli. Post-mortem examination revealed tuberculosis of the intestines, liver, spleen, and kidney. It is clearly possible that, with tuberculosis so widely disseminated throughout the body, purpura had occurred as a direct consequence. It is unusual, however, for such a condition to cause death so rapidly in this way. In most of the cases reported in the literature the purpura had occurred over a period of several months and, sometimes, years before death. In the present case there was no capillary fragility. There was gross thrombocytopenia, and the possibility arises that it may have been caused by the drugs given, for purpura did not occur until two days after treatment had been started. No purpura had been present previously and there was no previous history of such episodes.—M. J. SMALL

Supplementary Data on the Determination of Active Isoniazid in the Blood Serum by the Method of Vertical Diffusion in the Presence of PAS (in French). J. GROSSET* and G. CANETTI. *Rev Tuberc (Par)*, May–June, 1960, 24: 633–647. (*Paris, France)

A supplementary study is presented of certain technical points in the method of vertical diffusion for determination of active isoniazid in the serum: (*1*) It is preferable to prepare isoniazid solutions for the control system from animal serum as the inhibitory effect of bidistilled water is more pronounced than are the same dilutions in serum; (*2*) The diffusion of isoniazid placed at the bottom of the test tube takes place within a few hours in Löwenstein-Jensen medium; (*3*) A detailed study of neutralization of PAS by para-aminobenzoic acid (PAB) was made in the presence and absence of isoniazid. In the absence of isoniazid, concentrations of PAB equal to or higher than concentrations of PAS neutralize completely the effect of PAS. In order to neutralize concentrations of PAS obtained during chemotherapy, high concentrations of PAB are necessary (in the range of 500 γ per ml.). Neutralization of PAS is satisfactory in the presence of isoniazid if concentrations of PAB are higher than those of PAS. It is probable, however, that high concentrations of PAS, even if neutralized by 500 γ of PAB per ml. can in some cases lead to overestimation of the concentration of active isoniazid in the serum. For current practice these facts are without importance (after authors' summary).—V. LEITES

Phenylbutazone and Isoniazid in Pulmonary Tuberculosis. R. DESMEULES*, R. DION, F. MOISAN, and C. TURMEL. *Canad Med Ass J*, June 11, 1960, 82: 1219–1222. (*Laval Hosp., Ste. Foy, Quebec, Canada)

Twenty patients with moderately or far advanced pulmonary tuberculosis were treated with isoniazid and phenylbutazone, 400 mg. daily in divided doses. Four patients also received streptomycin. Seventeen of the patients were re-treatment cases. The duration of treatment was between 1 and 12 months. Cough and sputum were reduced in 10 patients. In 5 cases the sputum became negative for tubercle bacilli. Slight roentgenographic improvement was noted in 4 cases, and marked improvement in 2. Only 1 side reaction was noted, namely, nausea. Eight patients were affected, but in no instance was this reaction serious enough to warrant change of dosage.—E. A. RILEY

Combined Use of Nystatin and Antituberculous Drugs in the Management of Coexistent Fungal-Tuberculous Infections. G. ANGIOLI* and Z. GORECKI. *Canad Med Ass J*, June 18, 1960, 82: 1274–1275. (*Montreal, Canada)

Twenty-three patients with chronic pulmonary tuberculosis and evidence of coexistent fungal infection (in most instances Candida) were given combined therapy consisting of 1 or more antituberculous drugs and nystatin. Good clinical and good-to-marked roentgenographic improvement of the tuberculosis was recorded in 16 patients during the period of observation: 11 of 13 patients who were initially positive for tubercle bacilli became negative, while 10 patients initially negative remained so. Fungi were eliminated from the bronchial washings of 18 of the 23 patients. Fourteen of the 18 from whom fungi were cleared had previously harbored Candida species. Four of the 5 patients not cleared of fungi were among those with Candida species. No evidence of intolerance or undesirable reactions to nystatin was noted. These limited observations suggest that the oral administration of nystatin together with antituberculosis therapy in pulmonary disease with coexistent fungal infection offers a much improved prognosis, particularly when the fungal complication is due to species of Candida (after authors' summary).—E. A. RILEY

Continued Studies on the Problems of PAS Dosage. H. O. BANG and E. STRANDGAARD*. *Acta Tuberc Scand*, No. 2, 1960, 39: 81–96. (*Skorping Sanatorium, Denmark)

Nine or 12 gm. of calcium PAS granulate or 12 gm. of sodium PAS in solution were administered as a single massive daily dose to 115 tuberculous patients. Most patients preferred this mode of administration to the previous method of divided doses. The PAS blood concentrations were studied in 462 specimens from 39 patients. After administration of the single massive dose, PAS blood concentrations increased rapidly to between 10 and 20 mg. per 100 ml., then fell rapidly. Eight to 12 hours

elapsed, however, before blood concentrations dropped below 2 to 3 mg. per 100 ml.
—W. J. Steininger

Bioassay of Theophylline Preparations Using Human Subjects. B. Calesnick*, J. C. Munch, J. R. Di Palma, and V. R. Altarelli. *Brit Med J*, July 2, 1960, No. 5191: 33–36. (*Hahnemann Med. College, Philadelphia, Penn.)

When single doses of 4 different theophylline preparations were administered to normal individuals, no statistically significant difference could be detected in blood concentrations from 120 to 240 minutes. The adrenalin index revealed that the clinical effectiveness of various theophylline preparations depends on the absolute amount of theophylline present. Large doses produce an increase in duration of action rather than intensity of action (Authors' summary).—E. A. Riley

The Medical Letter*. (*136 E. 57th St., N. Y.)

October 28, 1960, 2: 85–88. Oxymorphone (Numorphan®), or 14-hydroxydihydromorphinone, as an analgesic. A new topical steroid, dichlorisone acetate (Diloderm®). Present evaluation of meprobamate (Miltown®).

November 11, 1960, 2: 89–92. A new vaccine combining polyvalent influenza virus with adenovirus types 3, 4, and 7 (Resprogen®); general use of adenovirus vaccines. Drug treatment of hyperthyroidism. A combination of nicotinic acid and aluminum hydroxide (Nicalex®) in hypercholesterolemia.

PULMONARY PHYSIOLOGY

The Clinical Usefulness of Routine Tests of Pulmonary Function. D. V. Bates*, J. A. P. Pare, and J. F. Meakins. *Canad Med Ass J*, July 30, 1960, 83: 192–197. (*McGill U., Montreal, Canada)

An evaluation is made of the work done by a pulmonary function study unit during its first 2 years of operation in a 500-bed general medical and surgical hospital. Tests included the measurement of: (*a*) indirect maximal breathing capacity by a single-breath method, combined with calculation of the maximal mid-expiratory flow rate; (*b*) functional residual capacity, subdivisions of lung volume, and mixing efficiency by closed-circuit helium; (*c*) resting diffusing capacity by the end-tidal sampling method; (*d*) arterial oxygen saturation and pCO_2 of arterial blood; and (*e*) exercise evaluation of oxygen uptake, ventilation, and diffusing capacity. In 47 patients studied, 37 of whom had advanced emphysema, the studies confirmed an obvious clinical diagnosis and/or made no contribution to management. In 137 patients studied, the tests contributed something to the clinical management. In 149 cases the tests contributed greatly to management and/or precise diagnosis. In 68 cases the tests were essential to accurate diagnosis and/or proper management. It is clear that a routine pulmonary function laboratory of the type described makes a major contribution to management or diagnosis in about 100 patients a year, fully justifying its use and establishment. Pulmonary emphysema was the principal problem among 153 patients studied.—E. A. Riley

Normal Standards for Single-Breath Tests of Ventilatory Capacity in Chidren. B. Gandevia*. *Arch Dis Child*, June, 1960, 35: 236–239. (*Royal Children's Hosp., Melbourne, Australia)

Tests of ventilatory capacity based on the recording of a maximal forced expiration after a full inspiration are becoming widely used. They are quicker to perform and require less cooperation from the patient then the conventional maximal breathing capacity test. They provide indices of: (*1*) forced vital capacity, (*2*) the volume of gas expired over a given time interval (0.5–1 second), and (*3*) the percentage of the vital capacity expired within the same time period. This method, which is fully described, was used on 170 children aged 5–14 years, and the results are tabulated and are related to standing height.—J. Lorber

Abnormalities of Ventilatory Capacity in Children with Asthma and Bronchiectasis. L. B. Strang*. *Arch Dis Child*, June, 1960, 35: 224–231. (*U. of Durham, England)

Serial readings of the forced expiratory volume were made on 59 children with chronic pulmonary disease and the results were compared with those obtained on 418 healthy school children. Serial recordings were performed on 20 asthmatic children (237 examinations) and on 39 children with bronchiectasis (209 examinations). Grossly abnormal records were found in both groups; these were related to the severity of the condition as judged by clinical and roentgenographic criteria, and the results are analyzed by statistical methods. The techniques of the experiments are fully described. These measurements provide an objective means of assessing an important aspect of these conditions which cannot be accurately assessed on clinical examination.—J. Lorber

Measurements of Pulmonary Diffusing Capacity in Children. L. B. Strang*. *Arch Dis Child*, June, 1960, 35: 232–235. (*U. of Durham, England)

Inhalation of low concentrations of carbon monoxide is used for the measurement of pulmonary

diffusing capacity in children. Using the method of Bates *et al.* (*J Physiol*, London, 1955, *129*, 237), the author examined 79 normal children, 37 with bronchiectasis, 15 with asthma, and 2 with diffuse pulmonary disease. A good significant correlation was found between the diffusing capacity for CO and the height, weight, and surface area in normal children. In bronchiectatic children the results were rarely outside the normal range, but there was a trend toward lower values. There was no correlation between the severity of the bronchiectasis and the diffusing capacity for CO. Asthmatic children and 1 child with mucoviscidosis gave normal results, while 1 child with pulmonary hemosiderosis had a severe impairment of diffusing capacity.—J. LORBER

Effects of Pharmacologic Agents on the Pulmonary Circulation. N. FOWLER*. *Amer J Med*, June, 1960, 28: 927–942. (*U. of Cincinnati, Ohio)

There are difficulties in interpreting drug-induced alterations in pulmonary blood flow and pressure in terms of changes in pulmonary vascular resistance. A major source of such difficulty is the distensibility of the pulmonary vessels, allowing them to respond passively to pressure and flow alterations. Drug-induced alterations in bronchomotor tone and effective blood viscosity also may lead to error. There is good evidence, obtained from isolated and perfused lungs of animals as well as from studies in intact animals and patients, that the pulmonary vasculature may respond to drugs independently of the systemic circulation. The composite effect of a drug on the pulmonary circulation may be greatly modified by its effect on the systemic circulation. The majority of studies indicates that hypoxia increases flow resistance in the pulmonary circulation, but not greatly. Breathing high carbon dioxide gas mixtures seems to temporarily increase flow resistance in the pulmonary circulation of the perfused lung, but it does not do so persistently in intact animals or man. Acetylcholine, infused directly into the pulmonary artery, appears to decrease pulmonary vascular resistance in a substantial percentage of patients with pulmonary hypertension associated with congenital heart disease or rheumatic heart disease. Serotonin appears to be a potent pulmonary vasoconstrictor. Studies with 20 per cent sodium chloride solutions and hypoxia suggest that pulmonary venous constriction may be an important mechanism in drug-induced pulmonary hypertension.—T. H. NOEHREN

Arterial Oxygen Unsaturation and the Ventilation-Perfusion Defect of Laennec's Cirrhosis. T. RODMAN*, M. SOBEL, and H. P. CLOSE. *New Engl J Med*, July 14, 1960, 263: 73–77. (*V. A. Hosp., Philadelphia, Penn.)

Studies of pulmonary function, arterial oxygen saturation, and ventilation-perfusion relations were performed in 56 patients with far advanced Laennec's cirrhosis and 1 patient with extrahepatic portal-vein thrombosis. A tendency to arterial oxygen unsaturation and increased venous admixture was demonstrated. In 10 of 20 patients without evidence of significant lung disease subjected to detailed pulmonary function and ventilation-perfusion studies, there was an unexplained respiratory alkalosis characterized by an increased minute ventilation, reduced arterial carbon dioxide content, normal or slightly elevated arterial pH, and reduced arterial carbon dioxide tension.—M. J. SMALL

Respiration and Circulation in Patients with Portal Cirrhosis of the Liver. H. O. HEINEMANN*. *Circulation*, July, 1960, 22: 154–159. (*College of Physicians and Surgeons, Columbia U., N. Y., N. Y.)

In addition to changes in the hepatic and splanchnic vasculature, which in turn lead to reduced hepatic blood flow, increased portal venous pressure, and the development of collateral vessels permitting bypass of the diseased liver, cirrhosis of the liver also may be associated with changes in the respiratory pattern, the pulmonary vasculature, and the systemic circulation. As a working hypothesis for future studies, the changes observed in adults with portal cirrhosis can be separated into 3 different groups. First, communications between the portal vascular bed and either the pulmonary veins or the venae cavae may: (*a*) permit venous blood to enter the systemic circulation and cause hypoxemia, or (*b*) allow intestinal metabolites to escape hepatic inactivation and gain direct access to the systemic circulation. Second, metabolites escaping hepatic inactivation may be responsible for changes in the pulmonary vessels, pulmonary hypertension, reduced peripheral resistance, and hyperventilation. Finally, reduction of the peripheral resistance leads to a rise in cardiac output and perhaps the plasma volume as well. The increased cardiac output may eventually cause cardiac hypertrophy and heart failure, a complication difficult to recognize in older patients in whom heart disease may occur independently, and fluid retention and dyspnea can be attributed to the underlying disease.—W. J. STEININGER

A Study of Gastric Secretions in Chronic Obstructive Pulmonary Emphysema. L. ZASLY*, G. BAUM, and J. RUMBALL. *Dis Chest*, July, 1960, 38: 69–72. (*V. A. Hosp., Coral Gables, Fla.)

Significantly increased gastric acidity was dem-

onstrated in this group of unselected, fasting patients with pulmonary emphysema. The hypothesis that carbon dioxide retention is implicated in this phenomenon could not be substantiated in this series. A possible stress mechanism is discussed. —E. A. Rouff

Studies of the Ventilation-Perfusion Relationships in the Lungs of Subjects with Chronic Pulmonary Empsysema, Following a Single Intravenous Injection of Radioactive Krypton (Kr^{85}). I. Presentation and Validation of a Theoretical Model. H. P. Gurtner*, W. A. Briscoe, and A. Cournand. *J Clin Invest*, July, 1960, 39: 1080–1090. (*Columbia U. College of Physicians and Surgeons, N. Y., N. Y.)

The behavior of intravenously injected Kr^{85} in the emphysematous patient is considered in terms of a model composed of 3 compartments: well-ventilated alveoli, poorly-ventilated alveoli, and tissues. Using typical emphysematous values for the volume, ventilation, and blood flows of the 2 lung compartments, the behavior of Kr^{85} in the various gases and blood streams is predicted when the effective tissue volume is either 10 or 60 L. Further calculated examples show the predicted effect of changes, during a study, in the distribution of blood flow to the lungs. Data are presented on the findings in 2 patients with emphysema in whom Kr^{85} concentrations were followed in expired air, arterial blood, and mixed venous blood after intracardiac injection of Kr^{85}. The behavior of the patients resembled that predicted for the model; the differences are discussed. Two approximate methods for determining the distribution of perfusion in the lung are presented. One depends only on expired gas analysis and the other on expired gas and arterial blood analysis. When tested on the predicted curves these appear to be in error by about 10 per cent.—E. Dunner

Cardiopulmonary Physiology in a Case of Pulmonary Alveolar Proteinosis. T. Snider*, F. Wilner, and B. Lewis. *Ann Intern Med*, June, 1960, 52: 1318–1328. (*Wayne State U. College of Med., Detroit, Mich.)

A case is presented of pulmonary alveolar proteinosis, a disease which has only recently been described in the literature. The roentgenogram was quite typical, with soft, finely nodular infiltrates radiating from the hila bilaterally to the medial, inferior portion of the lungs. Hilar nodes were characteristically not enlarged. The microscopic pathology consisted of relatively normal alveoli filled with a proteinaceous, PAS-positive material, probably produced from degenerated septal cells. There was no cellular infiltration of the alveolar septa. In this case, pulmonary function studies and right heart catheterization place this disease in the alveolar-capillary block group, with decreased total vital capacity, pulmonary compliance and pulmonary diffusing capacity, and a decrease in arterial oxygen saturation on exercise. No etiologic agent has as yet been identified as the cause of the disease, and no specific therapy has been found. Antimicrobials and steroids seem to have no effect on this condition, which may either regress spontaneously or progress to complete incapacitation and death due to secondary infection or right heart failure (after authors' summary).—T. H. Noehren

The Dynamics of Sulfur Dioxide Inhalation. O. Balchum*, J. Dybicki, and G. Meneely. *AMA Arch Industr Health*, June, 1960, 21: 564–569.(*U. of S. California School of Med., Los Angeles)

The combustion of coal and fuel oil has resulted in a considerable contamination of the atmosphere by sulfur dioxide (SO_2), amounting to as much as 2,000 tons per day in a large city such as New York. The effects of the chronic inhalation of low concentrations of this substance are of particular importance, as urban atmospheric concentrations usually do not exceed 0.5 to 1 ppm. Sulfur dioxide is readily absorbed from the upper and lower respiratory tracts even when inhaled in low concentrations such as 1 ppm. It can be absorbed by the tracheal mucosa when the latter alone is exposed to a degree sufficient to influence pulmonary function detrimentally. After absorption from the sites of deposition in the trachea, bronchi, and lungs, it is distributed to all tissues, including the brain. It can still be detected in the trachea and lungs 1 week after exposure. The physiologic and metabolic effects of the prolonged inhalations of low-level concentrations of SO_2 are unknown, and experiments are now in progress in an attempt to obtain further data on this aspect of the effects of inhalation of low concentrations of sulfur dioxide. —T. H. Noehren

Pulmonary Vascular Volume, Resistance, and Compliance in Man. W. R. Milnor*, A. J. Jose, and C. J. McGaff. *Circulation*, July, 1960, 22: 130–137. (*Johns Hopkins U. School of Med., Baltimore, Md.)

In 19 patients with a variety of cardiac and pulmonary abnormalities, pulmonary blood volume was measured with use of a dye-dilution method and simultaneous bilateral cardiac catheterization. The average pulmonary blood volume was 365 ml. per square meter of body surface (with a minimum of 126 and a maximum of 598 ml.). This value is significantly smaller than previous estimates by less direct methods. The ratio of pulmonary blood volume to mean intravascular pressure

was used as an indication of pulmonary vascular compliance. Patients with high pulmonary vascular resistance had relatively low pulmonary vascular compliance. A significant correlation between pulmonary blood volume and stroke volume was found, lending support to the hypothesis that the volume and elasticity of the pulmonary vascular bed contribute to the regulation of cardiac output. —W. J. STEININGER

Timed Vital Capacity: A Correlation with Maximal Breathing Capacity and Use as a Clinical Test of Ventilatory Function. G. C. LEINER*, M. J. SMALL, and S. ABRAMOWITZ. *Brit J Dis Chest*, July, 1960, 54: 210–216. (*V. A. Hosp., East Orange, New Jersey)

The literature on timed vital capacity is reviewed. In 400 patients with various chronic pulmonary diseases the one-second timed vital capacity was compared with the maximal breathing capacity. It was found that the determination of the one-second timed vital capacity with the aid of the Gaensler-Collins vitalometer is an easy and reliable method. A good correlation exists between the one-second timed vital capacity and the maximal breathing capacity. When the subjects are grouped as "normal," and as patients with "restrictive," "obstructive," and "combined" ventilatory defects, the ratio: M.B.C., L. per minute/one-second timed vital capacity, L., differs significantly between the groups, except between the "obstructive" and "combined" groups. For clinical evaluation of ventilatory function, the determination of the one-second timed vital capacity appears to be satisfactory (after authors' summary). —M. J. SMALL

A Simplified Closed Circuit Helium Dilution Method for the Determination of the Residual Volume of the Lungs. G. MENEELY*, C. BALL, R. KORY, J. CALLAWAY, J. MERRILL, R. MABE, D. ROEHM, and N. KALTREIDER. *Amer J Med*, May, 1960, 28: 824–831. (*Vanderbilt U., Nashville, Tenn.)

A simple rapid version is described of the helium method for estimating functional residual capacity employing equipment which is not unduly expensive and which is useful for a variety of other tests of pulmonary function. The results are highly reproducible. Evidence that the method is not subject to serious systematic error is presented.—T. H. NOEHREN

Relative Respiratory Depressant Effects of Oxymorphone and Morphine. J. BELLVILLE*, L. ESCARRAGA, S. WALLENSTEIN, R. HOUDE, and W. HOWLAND. *Anesthesiology*, No. 4, 1960, 21: 397–400. (*Stanford Med. Center, Palo Alto, Calif.)

A double-blind, cross-over, controlled experiment was done to evaluate the respiratory effects of morphine and oxymorphone. Seven healthy volunteers were used, and the method is described in detail. Oxymorphone was 8.95 times as potent as morphine in terms of analgesic effects. It was found to be 14.8 times as potent as morphine in terms of respiratory depressant effects. It is concluded that when oxymorphone is given in analgesic doses comparable to morphine, it produces at least as much respiratory depression.—E. SHABART

Comparison of Effects of Phenazocine and Meperidine on Respiration. E. GREISHEIMER*, L. KRUMPERMAN, B. RUSY, and D. ELLIS. *Anesthesiology*, No. 4, 1960, 21: 370–373. (*Temple U. School of Med., Philadelphia, Penn.)

The purpose of the study was to compare the effects of phenazocine and meperidine on alveolar ventilation and carbon dioxide tension of alveolar air. Sixteen healthy cooperative adults were used. The method employed is described in detail and was set up so that each subject served as his own control. Both drugs decrease the sensitivity of the respiratory center to carbon dioxide, although this does not signify that the reception of other types of stimuli is equally depressed. Clinically, phenazocine sedates to a lesser degree than does meperidine. Only one of the many factors involved in the control of respiration was tested in this experiment. Excitatory afferent impulses partly overcome the depression of the sensitivity of the respiratory center to carbon dioxide. The actual end result is respiration stimulation. Alveolar carbon dioxide tension during normal breathing is elevated after both drugs are administered. —E. SHABART

Factors Affecting the Rapidity of Alteration of Nitrous Oxide Concentration in a Circle System. E. EGER*. *Anesthesiology*, No. 4, 1960, 21: 348–355. (*U.S. Army Hosp., Ft. Leavenworth, Kansas)

This study illustrates the rate of change of concentration of nitrous oxide in a circle system which occurs on induction. The method employed is described in detail in young patients undergoing various operative procedures. High inflow rates (10 LPM or more) of nitrous oxide are necessary to achieve rapid change of concentration. This is found to be especially so in cases in which induction was the longest portion of the procedure. After induction, the anesthetic level due to nitrous oxide could be well maintained with low flows (1 to 3 LPM). Large and more muscular persons had less rapid rises in concentrations. Decreasing the volume of the anesthetic systems increased the rapidity with which concentrations could be reached.—E. SHABART

Anaerobiasis in Normal and Asphyxiated Premature Newborns. B. Vedra* and J. Ulrych. *Acta Paediat (Uppsa)*, March, 1960, 49: 129–134. (*Institute for the Care of Mother and Child, Prague, Czechoslovakia)

The relationship was studied of the lactic acid levels in the peripheral and venous blood of the mother, and the umbilical venous and arterial blood of premature normal and asphyxiated newborns. A close relationship exists between the levels in the mother and fetus, suggesting a high degree of interaction. In view of the relative amounts of extracellular fluid of mother and fetus, this relationship can be explained only on the basis of maternal production. Some results from asphyxiated premature newborns suggest that anaerobiasis in the premature does not play a significant role in protection against anoxia (after authors' summary).—H. J. Simon

Rescue Breathing for the Partly Drowned. L. Buchanan*. *Med J Aust*, March 12, 1960, I: 405–412. (*Sydney, Australia)

This is a comprehensive study of the incidence, epidemiology, physiology, and therapy of drowning. Illustrations are included to demonstrate the different methods of handling the drowning person on land and in the water.—H. J. Simon

The Effect of Pneumonectomy and Lobectomy on the Pulmonary Diffusing Capacity in Man. T. K. I. Larmi*. *Ann Chir Gynaec Fenn*, Fasc. 2, 1960, 49: 169–183. (*Kirurgi, Helsinki, Finland)

The pulmonary diffusing capacity was determined with the carbon monoxide method before and after 17 pneumonectomies and 18 lobectomies. An average decrease of 6 ml./min./mm. Hg., or 32 per cent of the preoperative values, was found after pneumonectomies, and a decrease of 4.7 ml./min./mm. Hg., or 23 per cent, after lobectomies. The changes in diffusing capacity following extensive pulmonary resection were relatively persistent.—J. Haapanen

Sugar Metabolism in Patients with Reduced Pulmonary Function (in Japanese). H. Koyama*. *Clin Resp Organs (Tokyo)*, July, 1960, 15: 493–499. (*National Tokyo Sanatorium, Kiyose, Japan)

Determinations of the blood and urine levels of pyruvic, alpha-ketoglutaric, and lactic acids, as well as the fructose tolerance test, were made on 25 patients with pulmonary tuberculosis who had reduced vital capacities. The vital capacity was less than 40 per cent in 17, and 41 to 60 per cent of the expected values in 8 cases; 12 patients with vital capacity of 61 per cent or more served as controls. The fasting blood and urine levels of pyruvic, alpha-keto-glutaric, and lactic acids were nearly equal in patients with reduced and normal respiratory functions. When the glucose tolerance test was done by injecting 20 gm. of glucose intravenously, a more highly elevated blood level 1 hour after injection than existed before injection was observed only in the case of pyruvic acid. When the fructose tolerance test was made by injecting 4 gm. of fructose intravenously, the blood levels of pyruvic, alpha-keto-glutaric, and lactic acids and acid-soluble inorganic phosphorus 1 hour after injection were elevated more than 100 per cent above those of the fasting levels. The urinary excretion of these substances 2 hours after injection was also raised. The blood and urine levels of these substances 1 and 2 hours, respectively, after injection of fructose were higher in proportion to the reduced vital capacity of the recipient patients.—I. Tateno

The Use of THAM to Prevent Hyperventilation and Acidosis while Breathing Carbon Dioxide. G. L. Brinkman*. *Amer J Med Sci*, June, 1960, 239: 728–731. (*Henry Ford Hosp., Detroit, Mich.)

In a study of 3 persons with normally sensitive respiratory centers, it was shown that tris(hydroxymethyl)aminomethane (THAM) prevents the hyperventilation and acidosis which normally occur while breathing 5 per cent carbon dioxide. The mode of action of THAM is discussed and it is postulated that, in alkalosis, the respiratory center is more responsive to arterial pH than to arterial carbon dioxide tension.—W. J. Steininger

MICROBIOLOGY AND IMMUNOLOGY

The Significance of *Candida Albicans* in Human Sputum. G. L. Baum*. *New Engl J Med*, July 14, 1960, 263: 70–73. (*V. A. Hosp., Cincinnati, Ohio)

Sputum specimens from 55 patients, 34 hospital employees, and 30 healthy medical students were studied for the presence of fungi. Fifty-five per cent of the patients, 36 per cent of the hospital employees, and 20 per cent of the medical students yielded sputum specimens positive for Candida species. Approximately half of these species were identified as *Candida albicans*. One medical student produced sputum that yielded a nonpathogenic Cryptococcus. It is believed that the source of the Candida found in the sputum specimens was the mouth, and that little significance can be attached to the finding of Candida in the sputum in the diagnosis of candidiasis of the lung. It is believed in this laboratory and by others that the

only way in which a diagnosis of pulmonary candidiasis can be made is by demonstration of the organism histologically and culturally in a lung biopsy specimen obtained through a thoracotomy incision.—M. J. SMALL

Studies on Acid-fast Bacilli Other than *M. Tuberculosis* Isolated from Tuberculous Patients and Experimentally Infected Guinea Pigs. II. Experiments of Boiling-fastness, Cord Factor Formation, Neutral Red and Niacin Tests, and Susceptibility to Antimicrobials (in Japanese). K. TOYAMA*. *Kekkaku*, July, 1960, 35: 480–487. (*Hosp. of the Kitasato Institute, Tokyo, Japan)

Sixty-one strains which were considered nonpathogenic acid-fast bacilli (the test group) and 22 strains of human and bovine tubercle bacilli (the control group) were subjected to the above tests. The Kf values in the boiling-fastness test with Ziehl's staining were 4 to 5 in only 1 strain of the test group, while all of the control group had values of 4 to 5. Cord factor formation in the fluid culture medium was positive in 3.3 per cent of the test strains and in 72.7 per cent of the control strains while, when the bacilli were cultured on Kirchner's agar plate, 1.4 per cent of the test strains and 86.4 per cent of the control strains were positive. The neutral red test was positive in 41 per cent of the test and 68.2 per cent of the control strains. None of the test strains gave a positive niacin reaction, while 95.5 per cent of the control strains were positive. Of the test strains, 93 per cent were resistant to pyrazinamide, 79 per cent were resistant to viomycin, and 52 per cent were resistant to kanamycin. A review of these results showed that most of the author's test strains were nonpathogenic acid-fast bacilli, although some of the strains had results compatible with "atypical" (unclassified) acid-fast bacilli. The niacin test was considered to be the best test for laboratory differentiation of these 2 groups of bacilli, although combination with boiling-fastness, cord formation, and neutral red tests appeared to be more complete.—I. TATENO

The Effect of Trace Metals on the Growth and Metabolism of *Mycobacterium Tuberculosis Avium*. D. S. P. PATTERSON*. *Tubercle*, June, 1960, 41: 191–202. (*Central Veterinary Lab., New Haw, Weybridge, Surrey, England)

The cultivation and metabolism of *M. tuberculosis avium* on synthetic media have been studied with particular reference to the trace-element status of the media. The effect of cobalt and/or zinc ions is to stimulate growth, the utilization of medium glycerol, and the production of coproporphyrin III. The stimulatory effect of zinc ions was examined in some detail and it was found to be dependent upon the presence of sufficient iron in the medium. With no added iron, 2–64 p.p.m. Zn^{2+} had no effect but, with as little as 2 p.p.m. Fe^{2+}, almost maximal stimulation of growth, glycerol utilization, and coproporphyrin production was observed. A given supplement of 4 p.p.m. Zn^{2+} stimulated the growth of a 46-day culture about 4 times, glycerol utilization about 5 times, and coproporphyrin production about 100 times. It was therefore concluded that zinc had a specific effect on the production of free coproporphyrin. Another result of zinc supplementation is to modify the nitrogen metabolism of the bacillus. Organisms grown on supplemented media are richer in nitrogen and, when autoclaved in phosphate buffer pH 9.0, up to 4 times more tuberculoprotein is released than in the case of those grown on unsupplemented media.—M. J. SMALL

The "Dassie" Bacillus. N. SMITH*. *Tubercle*, June, 1960, 41: 203–212. (*Sir William Dunn School of Pathology, Oxford, England)

Some aspects are described of the morphology, growth requirements, and resistance of a Mycobacterium isolated from an epithelioid granuloma in the lungs of the Cape hydrax or "Dassie," a genus of rodent common to South Africa. The morphology and staining features of the Dassie bacillus resemble closely those of *Mycobacterium* var. *muris* or the vole bacillus. Both organisms grow on Dorset egg medium without glycerin. A modified Dubos medium, in which the casein digest is replaced by 1-glutamic acid–sodium salt at 0.1 per cent, is a satisfactory medium for growth of the Dassie bacillus. This bacillus was found to be susceptible to both streptomycin and isoniazid. —M. J. SMALL

The Lipids of Weybridge PPD. D. S. P. PATTERSON*. *Tubercle*, June, 1960, 41: 186–190. (*New Haw, Weybridge, Surrey, England)

The preparation of acetone- and ether-dried powders from Weybridge PPD was accompanied by the loss of about 10 per cent of the weight of the resulting powders as lipid material which dissolved very readily in these organic solvents. The residual lipid, most of which was rather firmly bound to the tuberculoprotein, could be removed successively with a chloroform-methanol mixture and with acidified ether-ethanol. The surface activities were measured of different tuberculoprotein preparations in aqueous solution. Those containing high proportions of lipid were the most active. The lipid fraction apparently increases the tendency of tuberculin to be adsorbed at glass/liquid interfaces and is, therefore, partly responsible for the so-called "volume effect" which is ob-

served in stored dilutions of tuberculin. Another possible function of the lipid moiety is to minimize the destruction of the biologic activity of tuberculin at elevated temperatures (after author's summary).—M. J. Small

EXPERIMENTAL PATHOLOGY

Chronic Pulmonary Emphysema (An Experimental Study). II. Spontaneous Pulmonary Emphysema in Rabbits. H. T. G. Strawbridge*. *Amer J Path*, September, 1960, 37: 309–331. (*U. of Liverpool, England)

An account is given of the types of spontaneous chronic vesicular emphysematous lesions and associated inflammatory changes found in the lungs of 155 rabbits. The emphysematous lesions and the elastica were studied in paraffin sections 100 μ thick. The microscopic appearances of all types of emphysema were essentially the same and consisted of the development of progressive fenestration of the alveolar walls leading to destruction of the walls. The elastic tissue showed no changes which antedated the appearance of fenestration, and seemed to be the most resistant component of the alveolar framework. The appearances are considered to be the result of an atrophy of the alveolar walls.

The following types of chronic emphysematous lesions have been distinguished: (*1*) Generalized emphysema: This can only be diagnosed, with certainty, microscopically. It is not the direct result of inflammatory changes, but there may be an indirect relationship. The incidence of generalized emphysema is nil in young rabbits, but rises to more than 50 per cent in rabbits older than 2½ years. Various acute operative procedures did not influence the incidence of generalized emphysema. (*2*) Emphysema in marginal lobules: Marginal lobules are formed by inflammatory deformity of the sharp margins of the lungs. Initially, the lobules are not emphysematous but become so subsequently. This type of emphysema should be disregarded in assessing the results of attempts to produce experimental emphysema. (*3*) Vesiculation: These minute marginal emphysematous lesions usually constitute merely a local accentuation of generalized emphysema. But some examples may be the result of local inflammatory changes, as in the case of marginal lobules. Therefore, caution is needed in the assessment of the significance of vesiculation in the experimental production of emphysema. The associated inflammatory lesions were those of a subacute or chronic interstitial pneumonia, the causative organism of which was not identified (after author's summary).

A Profile of Electrolytes in the Cartilaginous Plate of Growing Ribs. D. S. Howell*, E. Delchamps, W. Riemer, and I. Kiem. *J Clin Invest*, July, 1960, 39: 919–929. (*U. of Miami School of Med., Fla.)

A method has been described by which quantitative electrolytic determinations on pooled calf-rib costochondral junctions may be correlated with the histologic structure of the epiphyseal plate. In comparison with resting cell tissue, the hypertrophic and proliferating cell zones showed a four- to sixfold greater relative cell volume, threefold higher potassium, twofold greater sulfur content, twofold larger amount of sodium in excess of the theoretic equivalent associated with chloride, 50 per cent smaller content of collagen nitrogen, variably elevated levels of calcium and phosphorus due to hydroxyapatite deposition, two- to fivefold higher levels of calcium and phosphorus due to hydroxyapatite deposition, and two- to fivefold higher levels of magnesium, carbonate, and citrate. Chloride content changed only slightly across these zones. The method should prove useful in enzymatic, electrolytic, and thermoelastic studies of growing cartilage (author's summary).—E. Dunner

Some Factors Altering the Severity of Acute Radiation Pneumonitis: Variation with Cortisone, Heparin, and Antibiotics. W. T. Moss*, F. J. Haddy, and S. K. Sweany. *Radiology*, July, 1960, 75: 50–54. (*Chicago Wesley Memorial Hosp., Illinois)

The effect of steroids, heparin, and antimicrobials upon radiation lung damage was studied in rats. Total chest compliance (the relation of pressure to volume) was selected as the measure of damage. Irradiation decreased compliance. This change was unaffected by heparin, but was questionably prevented by antimicrobials. Early, prolonged cortisone therapy definitely reduced the severity of acute radiation-induced lung damage. When cortisone was started three weeks after irradiation and after lung changes were obvious, the benefit was less striking.—W. J. Steininger

The Relationship Between Oxygen Tension of Inhaled Gas and the Severity of Acute Radiation Pneumonitis. W. T. Moss* and F. J. Haddy. *Radiology*, July, 1960, 75: 55–58. (*Chicago Wesley Memorial Hosp., Illinois)

In experiments with rats, it has been shown that total thoracic compliance (relation of pressure to volume) is decreased by irradiation. The change is less severe if the animals are irradiated at high oxygen tensions. The adrenals are heavier in the high oxygen than in the low oxygen group of animals. It is suggested that the severe skin and marrow reaction produced in the high oxygen

group results in adrenal hyperplasia with increased production of endogenous cortisone and the subsequent suppression of compliance changes.
—W. J. STEININGER

Effects of Ultramicroscopic Gamma-Aluminum Oxide on Rats and Mice. W. KLOSTERKOTTER*. *AMA Arch Industr Health*, May, 1960, 21: 458–472. (*Hygiene-Institut der Westfälischen Wilhelms-Universität, Munster, Germany)

These investigations were an attempt to clarify the questions of whether exposure of workers to gamma-aluminum oxide (γ-Al_2O_3) can be expected to cause lung damage, whether the γ-Al_2O_3 portion of bauxite furnace fumes presents a lung hazard, and whether it contributes essentially to the development of Shaver's disease. The tissue effect of 5–40 mu of γ-Al_2O_3 dust has been investigated in experiments on mice and rats. In the case of massive local accumulations (peritoneal test on the rat and mouse) the γ-Al_2O_3 damages the cells and has a strongly fibroplastic effect. After intratracheal injection of 35 mg. in rats, the tissue reaction reaches the II and III Degrees, according to Belt and King. High dose rate and long duration of inhalation in the case of rats cause heavy desquamation of the alveolar cells and secondary inflammation processes. Cell damage and fibrous tissue formation remain relatively small in the pulmonary dust foci (I and I–II Degrees according to Belt and King). The regional lymph nodes exhibit no fibrosis in the case of heavier Al_2O_3 accumulation. Progressive fibrosis with development of cell-free collagenous-hyaline nodules, such as is typical for quartz, was not observed in any case. Smaller inhalations of submicroscopic γ-Al_2O_3 may be considered to be relatively harmless; there is a definitely pathogenic effect at higher concentration. The causative role of γ-Al_2O_3 in the occurrence of corundum smelter's lung cannot be dismissed. However, in animal experiments its effect is less fibroplastic and significantly less toxic than silicon dioxide (SiO_2) fumes. Thus, apparently, the greater significance attaches to amorphous SiO_2.—T. H. NOEHREN

***Rev Tuberc* (*Par*)**, May–June, 1960, 24: 589–632:

Hypersensitivity of the "Retarded Type" to a Lipopolysaccharide of *M. Tuberculosis*. A Comparative Study of Intradermal Tests to Tuberculin and to This Nonprotein Fraction (in French). N. CHOUCROUN*, P. GRASSLAND, and R. KOURILSKY. Pp. 589–604. (*Paris, France)

A Study of Variations in Intensity of Delayed Hypersensitivity Reactions to the Polysaccharide PmKo and to Tuberculin in Correlation with the Clinical and Anatomic Course of Tuberculosis (in French). Pp. 605–632.

A lipopolysaccharide fraction of *M. tuberculosis*, PmKo, identified in 1940 as a polysaccharide of mycolic acid, has antigenic properties not due to the presence of proteins, and plays an essential role in the development of tuberculin allergy. Two bacillary constituents, a tuberculoprotein and the lipopolysaccharide PmKo, are necessary to produce hypersensitivity to *M. tuberculosis*; each of the fractions separately is incapable of producing allergy. The characteristic reaction to PmKo has its maximal intensity after 24–48 hours; the induration is more persistent (2–4 weeks) than with tuberculin; localized ulcerations are more frequent. Comparative intradermal tests with tuberculin and PmKo were performed in 702 patients on a chest service. Patients who reacted to tuberculin also had positive reactions to PmKo and vice versa, but the intensity of the reactions varied. With equivalent doses of antigen the intradermal reaction to PmKo is usually more intense than the tuberculin reaction in old cases of tuberculosis with low-grade activity; in cases of old inactive tuberculosis the predominance of stronger reactions to PmKo is the rule. On the other hand, tuberculin reactions are generally more intense in acute caseous pulmonary tuberculosis. Reactions to both antigens are usually strong in caseous adenopathy; they are both weak in serofibrinous pleurisy. It is concluded that caseating inflammatory processes are generally accompanied by a strong tuberculin reaction which is of higher intensity than the reaction to PmKo. A tendency toward stabilization, progressive fibrosis, and calcification are accompanied by an increasingly strong reaction to PmKo, which seems to indicate the level of antituberculous resistance.—V. LEITES

Plasma Fibrinogen in the Differential Diagnosis of Pulmonary Diseases with Special Reference to Bronchial Cancer (in German). H. KEMNITZ*. *Z. Tuberk*, February, 1960, 114: 312–329. (*Lungenkrankenhaus, Leipzig, Germany)

The plasma fibrinogen level was determined in 120 healthy persons: it was higher in older age groups and slightly higher in females. The fibrinogen level was then determined in 237 patients with different pulmonary diseases. In cases of bronchial cancer it was considerably elevated; in exudative tuberculosis and progressive silicotuberculosis it was moderately or considerably elevated; in productive tuberculosis, normal or slightly increased; in silicosis, normal or slightly elevated; and in chronic pneumonia and in lymphogranulomatosis, it was normal. Thus the determination of plasma fibrinogen seemed to be a valuable addition to diagnostic procedures.
—J. HAAPANEN

PUBLIC HEALTH AND EPIDEMIOLOGY

Tuberculosis in China. Leading Article. *Tubercle*, April, 1960, 41: 148–149.

This article summarizes and comments on an account by the Antituberculosis Association of China. There were only 12 institutions devoted to tuberculosis before 1949, with fewer than 100 physicians and a little more than 600 beds. At present there are 101 antituberculosis clinics with 4,306 beds, 61 tuberculosis hospitals with 16,455 beds, and 74 sanatoriums with 14,715 beds. Much emphasis has been placed on prevention and health education with films, publications, posters, broadcasts, and plays. The work was begun among workers in the factories, mines, state organizations, and urban schools and extended by degrees to rural areas. BCG vaccination has been widely used. The incidence and mortality of tuberculosis in children has been greatly reduced. Among a population of 80,000 in Chiansing, about 32,600 vaccinations were done from 1951 to 1958; 4 children died of tuberculous meningitis in 1952, 3 in 1953, and there were no deaths in the next 5 years. The mortality in Peking is said to have fallen from 22.9 to 4.6 per 10,000. The decline is attributed to the rise in living standards and improvement of working conditions, with improved sanitation and personal hygiene, prompt treatment, and enforced vaccination. Chemotherapy is used for long periods and isoniazid–PAS is the most common combination, with streptomycin or other drugs for advanced cases. Collapse therapy is still commonly employed and considered to be a valuable procedure in association with chemotherapy. Instillation of drugs directly into cavities through a fine endotracheal catheter has been used with good effect at the Fu Wai Hospital. Studies at the Peking Tuberculosis Clinic and Municipal Tuberculosis Hospital suggest that intrathecal injections of streptomycin are no longer necessary in treating tuberculous meningitis. Surgical treatment has developed rapidly in the past 10 years. Conservative treatment of bone and joint tuberculosis has been abolished in favor of chemotherapy and focal debridement. In the Peking Tuberculosis Institute this is done by the transpleural route in cases of tuberculosis of the thoracic vertebrae.

Of particular interest to "Western" readers is the combinaton of traditional Chinese medicine with modern chemotherapy and surgery. Many traditional drugs have been studied in the laboratory and in clinical practice. Animals treated with *Podophyllum versipelle* are reported to have shown "improvement in pathogenicity index, splenic index and survival period." Traditional prescriptions with *Bletilla hyacinthina* and *Stemona sessilifolia* have been tested at the Tuberculosis Prevention and Treatment Centre in Liaoning, and sputum conversion was reported in 41 per cent of 47 patients after 3 months' treatment, with closure of cavities in 8 of 20 cases. The Peking Research Institute of Tuberculosis has studied the effect of berberine and reported roentgenographic improvement in more than ⅓ of the cases so treated. From Peking too it has been reported that treatment with moxibustion and chemotherapy gave better results than were observed in a control group treated with drugs alone. It is stated that traditional medicine has also made important contributions to the treatment of bone tuberculosis. It would be foolish to dismiss all traditional remedies as completely valueless, and it is interesting that some of the Chinese remedies with the best reputations are being studied in tuberculosis research centers in that country.—M. J. Small

A Clinical and Environmental Study of Byssinosis in the Lancashire Cotton Industry. S. A. Roach* and R. S. F. Shilling. *Brit J Industr Med*, January, 1960, 17: 1–9. (*London School of Hygiene and Tropical Med., England)

The prevalence of byssinosis was measured in a worker population employed in 3 coarse- and 2 fine-cotton mills, and their disabilities were graded by symptoms. Dust concentrations were measured with a dust-sampling machine based on the hexhlet (a dust sampler first described in this journal by Wright, B. M., 1954, *9*: 112). Well over half of the workers working in the card rooms of the coarse mills had symptoms of byssinosis, whereas less than 10 per cent of the workers in the card rooms of the fine mills were symptomatic. The mean dust concentrations ranged from 90 mg. per 100m³ in one section in a fine mill, to 440 mg. per 100m³ in a similar section in a coarse mill. The prevalence of byssinosis was closely related to the degree of dustiness. For the 3 main constituents of the dust, namely, cellulose, protein, and ash, the prevalence of byssinosis correlated most highly with that of protein, particularly with the protein in the medium-sized dust particles, i.e., approximately 7 microns to 2 mm. The symptoms of byssinosis may be caused by something in the plant debris which affects the respiratory tract above the level of the terminal bronchioles. This is the site in which the medium-sized dust deposits. The possible importance of the fine dust is discussed.

The authors suggest that, on the basis of their measurements, a mill with a dust concentration of 100 mg. per 100m³ or less would be safe. They state, however, that such concentrations are not at present achievable in dusty card rooms. Therefore, it is suggested that the level of dust at no

time be allowed to exceed 250 mg. per 100m³, and that periodic medical examinations be adopted to protect susceptible workers who can be advised to leave their dusty environment before they become permanently disabled.—H. J. SIMON

The Epidemiology of Primary Atypical Pneumonia. H. HARDING* and R. SNYDER. *AMA Arch Intern Med*, February, 1960, 105: 217–232. (*Evanston Hosp., Illinois)

In this report, experience with 116 cases of epidemic and sporadic primary atypical pneumonia is presented. Evidence for the possible existence of a subclinical type of the disease is also given. It again has been found that primary atypical pneumonia apparently is not due to any of the common bacteria nor the better known viruses or rickettsiae (authors' summary).
—T. H. NOEHREN

Some Practical Aspects of the Smoking-Cancer Problem. E. L. WYNDER* and D. HOFFMANN. *New Engl J Med*, March 17, 1960, 262: 540–545. (*Sloan-Kettering Institute, New York)

Some practical suggestions are given that should lead toward reducing the risk of cancer of the respiratory tract among smokers. The following measures are suggested: moderation of smoking for those who cannot give up the habit; use of filter cigarettes with the lowest yield of smoke condensate; avoidance of smoking the cigarette to the butt, since there is significantly more smoke condensate in the latter part of the cigarette; and, not inhaling deeply since such a practice leads to much greater absorption of smoke condensate by the lungs. The benzo[a]pyrene content of various cigarette-smoke condensates is similar when judged on a gram-for-gram basis. Per cigarette, however, it increases together with an increase in the amount of smoke condensate. Thus, the user of a cigarette with a high yield of smoke condensate will be exposed to more benzo[a]pyrene than one smoking a cigarette with a lower yield. Filter cigarettes tend to diminish symptoms, especially that of cough, often found to be associated with smoking. Further research must be carried out in an attempt to reduce carcinogens and co-carcinogens from tobacco-smoke condensate (after authors' summary).—M. J. SMALL

Transport of Streptococci on Filter Paper Strips. N. F. HOLLINGER*, L. H. LINDBERG, E. L. RUSSELL, H. B. SIZER, R. M. COLE, A. S. BROWNE, and E. L. UPDYKE. *Public Health Rep*, March, 1960, 75: 251–259. (*U. of California, Berkeley)

The filter paper strip technique proved to be highly satisfactory for transport and holding of throat swabbings for subsequent culture of beta hemolytic streptococci.—E. DUNNER

Present Health of Children Given X-Ray Treatment to the Anterior Mediastinum in Infancy. E. A. CONTI, G. D. PATTON, J. E. CONTI, and L. H. HEMPELMANN*. *Radiology*, March, 1960, 74: 386–391. (*U. of Rochester School of Med., New York)

Between 1938 and 1946, X-ray treatments to the thymus gland were routinely administered to 1,564 infants in Pittsburgh. Ninety-five per cent of the 1,340 children with thymus glands of normal size were given 150 r through a 4 × 4-cm. port. In 80 per cent of the 224 cases with thymic enlargement at birth, the radiation dose was 200 to 400 r (140 kvp) through a port of the same size. The medical histories of these children and their 2,923 untreated siblings were reviewed between 1956 and 1958. No cases of malignant disease were found in the treated group. The 4 malignant tumors in the sibling group, including 1 case of leukemia, compared closely with the expected number. The reduction of the incidence of cancer and leukemia in the treated group is not statistically significant. The incidence, however, is significantly below that in other reported series of cases treated with comparable doses of radiation through larger ports. It is suggested that the small port size in the present series, resulting in a reduction of the mass of irradiated tissue, could explain the apparent noncarcinogenicity of these exposures.—W. J. STEININGER

Motor Exhaust Gases and Lung Cancer in Cincinnati. C. A. MILLS*. *Amer J Med Sci*, March, 1960, 239: 316–319. (*U. of Cincinnati, Ohio)

Basic survey data show lung cancer death hazards to be greatest at all ages above 40 for drivers who are residents of Cincinnati's air-polluted Basin areas, slightly less for drivers residing in clean suburban areas, and utterly lacking for drivers residing far out in clean rural areas where driving is mostly on open country roads. In general, urban residents exposed to urban traffic for more than 12,000 miles per year exhibit more than twice the lung cancer incidence rates than those with lesser urban driving mileages. It is recommended that the medical profession give specific recognition to these urban driving hazards and to motor exhaust gases as the dominant factors in today's increasing development of the ozone-type smogs now recognized as community health hazards at ozone concentrations above 0.1 p.p.m.—W. J. STEININGER

Respiratory and Cardiac Deaths in Los Angeles Smogs during 1956, 1957 and 1958. C. A. MILLS*. *Amer J Med Sci*, March, 1960, 239: 307–315. (*U. of Cincinnati, Ohio)

Los Angeles County not only has almost 400 smog-correlated deaths a year, but there is also a direct and close relationship between daily maximal temperature and ozone (or oxidant) formation in its atmosphere. This close ozone–temperature relationship existed at temperatures ranging from 46°F. to 104°F. during the 3-year period 1956 through 1958. The excess deaths on smoggy days cannot logically be considered heat deaths except at temperatures above 96°F. in Los Angeles' low humidities. Therefore, the conclusion seems justified that ozone (or oxidant) smogs themselves are community health hazards at ozone levels above 0.1 p.p.m. To prevent atmospheric ozone levels above 0.1 p.p.m., automobile exhaust output of unburned hydrocarbons (the major source of ozone preformers) would have to be reduced by about 85 to 90 per cent from present levels. Tables are given of 3-year daily maximal temperatures, daily respiratory and cardiac deaths, and daily average maximal ozone (or oxidant) concentrations for all reporting Los Angeles County stations.—W. J. STEININGER

A Comprehensive Radiation Control Program in New York City. H. BLATZ*. *Radiology*, March, 1960, 74: 474–479. (*Office of Radiation Control, 386 Fourth Ave., N. Y., N.Y.)

The Radiation Code of New York City, in effect since June, 1958, requires registration of all sources of radiation within the city and requires compliance with recommendations of the National Committee on Radiation Protection. The program is twofold: (*1*) administration of the Code to make certain that radiation workers, medical and dental patients, and the general population are not being unnecessarily exposed routinely to radiation and radioactive materials, and (*2*) protection against adverse effects of radiation incidents and accidents. X-ray installations are inspected at 3 stages. At the first visit, the inspector checks for serious deficiencies, such as failure of X-ray collimation in chest and dental roentgenography, insufficient protective shielding for technicians, and improper leading of fluoroscope screens. The second stage of inspection consists of a further visit, if indicated, at which time fluoroscopic output is measured if the kilovoltage, milliamperage, tube-to-panel distance and filter could not be determined by inspection, plus any particularly critical measurements. The third stage is a general test of structural shielding. An extensive professional and public educational program has been initiated, including distribution of *Handbook 60* to all physicians and dentists, meetings with X-ray manufacturers and dealers, addresses to professional societies, and establishment of a technical reference library.
—W. J. STEININGER

Number and Distribution of Roentgenologic Examinations for 100,000 People. R. F. BROWN*, J. HESLEP, and W. EADS. *Radiology*, March, 1960, 74: 353–363. (*U. of California Med. Center, San Francisco)

Analysis was made of the roentgenographic examinations performed during 1956 and 1957 on 100,000 registrants of a total medical care health plan. On the basis of Laughlin and Pullman's estimated dose per examination, the total gonadal exposure per person, up to the age of 30 years, was calculated as 1.2 to 1.5 r. It is suggested that the basic data used in the present study are as reliable as those employed by the various authors for estimates published in 1956 by the National Academy of Sciences. This assumption would lead to the conclusion that the gonadal exposure of the United States population up to age 30 lies below the 4.5 r "probable average" widely used and quoted by those interested in radiation protection. A gonadal dose of less than 1.5 r during the course of 30 years is readily attainable without altering the present use of diagnostic roentgenography. In fact, if roentgenographic examinations are based on medical indications and the proper equipment and technique are used, an average gonadal dose of less than 500 mr in 30 years could be achieved.—W. J. STEININGER

Further Observations on the Frequency of *Mycobacterium Tuberculosis* var. *Bovis* in Pulmonary Tuberculosis in Humans (Note V) (in Italian). M. LA PLACA* and E. RASPI. *Riv Pat Clin Tuberc*, No. 4, 1959, 32: 503–507. (*Ist. Microb., U. of Bologna, Italy)

Only 2 bovine strains were found among 111 strains of *M. tuberculosis* isolated from a corresponding number of patients. The 2 bovine strains were recovered from 2 patients who, because of their work, had been often and for a long period of time in contact with cattle.—G. CASTELNUOVO

Relationship of Noxious Gases to Carcinoma of the Lung in Railroad Workers. I. KAPLAN*. *JAMA*, December 12, 1959, 171: 2039–2043. (*B. and O. Railroad Service, Baltimore, Md.)

A study of the medical records of deaths among railroad workers showed that the group of workers regularly exposed to diesel exhaust proved not to have any higher rate of bronchopulmonary carcinoma than did other groups such as clerks and janitors.—H. ABELES

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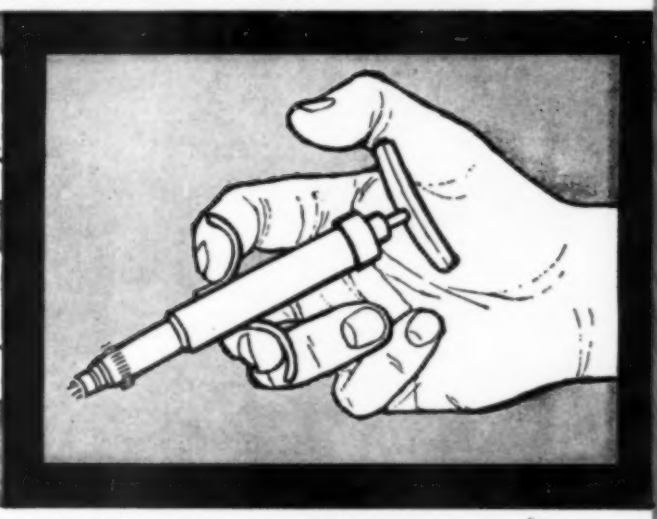

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